KU Leuven
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KU LEUVEN
DOCTORAL SCHOOL
BIOMEDICAL SCIENCES

NEURAL CONTROL OF WALKING: ROLE OF THE CEREBELLUM

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Dissertation presented
in partial fulfillment of the
requirements for the degree of
Doctor in Biomedical Sciences

December 2014

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December 2014

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Summary

Walking is a relatively easy task; we can walk without thinking about how to do it. While we are able to walk without thinking about it, from a control perspective, walking is definitely not a simple task. It needs to be stable and efficient in a broad range of different conditions. Insights into the neural control of gait are important to learn us about why specific individuals (patients, elderly) experience difficulties with walking, in general or under specific conditions. With this knowledge rehabilitation and fall prevention strategies can be optimized.

While it is known that there is an important role for the spinal circuitry in the neural control of gait, multiple supraspinal structures have been suggested to contribute substantially as well. Specifically, based on common gait deficits in cerebellar patients, the cerebellum can be expected to be important in the neural control of gait. So far this has been evaluated in humans, mainly during unperturbed, steady state walking. An important next step is to study the role of the cerebellum in the control of gait corrections in reaction to perturbations. In those conditions, the cerebellum can be expected to be even more involved in the control of gait, because of its function in comparing expected and real sensory signals.

The objective of this thesis was to increase the understanding of the roles of the cerebellum in the control of gait corrections in reaction to perturbations, and of the localization of these functions within the cerebellum. We evaluated the role of the cerebellum in dynamic gait stability, in cutaneous reflex modulation during gait and in locomotor adaptation, all important features of neural control of gait

in non-steady state conditions.

In the first part, we performed three studies, focusing on the relationship between cutaneous reflex modulation and gait stability, and on the role of the cerebellum in these features. First, we studied cutaneous reflexes during backward walking in healthy controls and observed a prominent phase-dependent reflex modulation during this task. Next, we addressed the potential role of the cerebellum in the control of cutaneous reflexes. Cutaneous reflex modulation was similar between healthy controls and patients with focal cerebellar lesions, but the latter appeared less able to attenuate reflexes to self-induced stimuli. This suggests that the cerebellum is not primarily involved in cutaneous reflex modulation but that it could act in attenuation of self-induced reflex responses. The latter role in locomotion would be consistent with the common view that the cerebellum predicts sensory consequences of movement. Furthermore, biceps femoris muscle activity during the single stance phases was increased in the patient group compared to the controls. This increased activation was likely related to a co-activation strategy to reduce instability of gait. This was supported by findings in our third study, where we evaluated dynamic gait stability in patients with focal cerebellar lesions and in healthy controls. The short-term maximum Lyapunov exponent was higher in cerebellar patients, indicating reduced dynamic gait stability. Furthermore, while step width was increased and self-selected overground walking speed was decreased in the patient group while other spatio-temporal gait parameters were similar. Patients with the largest lesions in the vermis displayed the least stable gait pattern.

In the second part, we focused on split-belt walking which, in the past decade, has become a popular paradigm to study the role of the cerebellum in locomotor adaptation. First, we evaluated split-belt adaptation in healthy controls and mildly ataxic patients with focal cerebellar lesions. We observed that during the split-belt adaptation experiment, patients and healthy controls globally displayed similar changes in gait parameters. However, a group difference was observed in the after-effect of the Stance Time Symmetry: during the early phase of the post-adaptation period the relative stance times were more asymmetric for the patient group than for the control group. Patients who walked with more asymmetric relative stance times were more likely to have lesions in vermal lobules VI and Crus II. In the final study, we assessed the role of somatosensory perception in the control of split-belt walking. We observed that participants who were less able to perceive differences between belt speeds, initially walked with more asymmetric stance times during split-belt walking. This is in line with our general view that load and stretch information are important in the neural control of gait.

In conclusion, the cerebellum appears important in the control in dynamic gait stability. Furthermore, our data suggests that the cerebellum is not primarily involved in cutaneous reflex modulation but that it could act in attenuation of self-induced reflex responses. Our results demonstrated that mildly ataxic cerebellar patients show no deficits in split-belt adaptation but exhibit differences in the post-adaptation period. Finally, the observed relations between speed-difference perception and gait asymmetry during split-belt walking confirmed the importance of proprioceptive information in gait control.

Samenvatting

Wandelen is relatief gemakkelijk, we doen het zonder er bij na te denken. Echter vanuit een besturingsperspectief is het verre van gemakkelijk. Onder een groot scala van omstandigheden moet het stabiel en efficiënt zijn. Inzichten in hoe ons zenuwstelsel wandelen aanstuurt kunnen ons helpen in te zien waarom sommige mensen (patiënten of ouderen) moeite hebben met wandelen, in het algemeen of enkel in bepaalde gevallen. Met deze kennis kunnen we revalidatieprocessen en valpreventie strategieën optimaliseren.

Naast de bekende belangrijke rol van het ruggenmerg in de neurale sturing van het wandelen, zijn er verschillende supra-spinale structuren die gedacht worden hier ook substantieel aan bij te dragen. Gezien het bekende verstoorde gangbeeld van cerebellaire patiënten, kan verwacht worden dat vooral het cerebellum belangrijk is binnen de neurale sturing van het wandelen. Tot nu toe, is dat bij mensen voornamelijk onderzocht tijdens onverstoord wandelen. Een belangrijke volgende stap is het bestuderen van de rol van het cerebellum in de neurale sturing van corrigerende bewegingen na verstoringen tijdens het wandelen. Het is namelijk te verwachten dat het cerebellum hierbij extra belangrijk zal zijn, gezien de cerebellaire functie in het vergelijken van verwachte en werkelijke sensorische signalen.

De doelstelling van deze thesis was om het begrip te verhogen van de rol van het cerebellum in de neurale sturing van corrigerende bewegingen na verstoringen tijdens het wandelen en van de lokalisatie van deze functies binnen het cerebellum. Hiervoor bestudeerden we de rol van het cerebellum in dynamische gangstabiliteit,

in modulatie van cutane reflexen tijdens het gaan en in gangadaptatie, allemaal belangrijke functies van neurale sturing van het wandelen in verstoorde condities.

In het eerste deel, voerden we drie onderzoeken uit, gericht op de relatie tussen cutane reflex modulatie en gangstabiliteit, en op de rol van het cerebellum daarin. Eerst onderzochten we cutane reflexen tijdens achteruit wandelen bij gezonde proefpersonen. Daarbij zagen we duidelijk dat de modulatie van cutane reflexen faseafhankelijk is. Vervolgens onderzochten we de mogelijke rol van het cerebellum binnen deze faseafhankelijke regulatie van cutane reflexen. Gezonde controle proefpersonen en patiënten met focale cerebellaire laesies vertoonden gelijkwaardige reflex modulatie, maar de patiënten leken wel minder in staat om reflexen na zelfgeïnduceerde stimulatie te verminderen. Dit suggereert dat het cerebellum niet primair betrokken is bij cutane relfex modulatie, maar wel een rol zou kunnen spelen bij het verminderen van zelfgeïnduceerde reflexen. Dat laatste zou goed aansluiten bij beeld dat het cerebellum de sensorische consequenties van bewegingen voorspelt. Voorts, de spieractiviteit van de biceps femoris tijdens de enkele standfase was hoger in de patiëntengroep dan in de gezonde proefpersonen. Deze verhoogde activiteit had waarschijnlijk te maken met een coactivatiestrategie om de ganginstabiliteit te verminderen. Dit idee werd ondersteund door de bevindingen in het derde onderzoek, waar we dynamische gangstabiliteit bij patiënten met focale cerebellaire laesies en gezonde controle proefpersonen onderzochten. De korte termijn maximale Lyapunov exponent was hoger in de cerebellaire patiënten, duidend op een verminderde gangstabiliteit. Verder was de stapbreedte groter en de voorkeurssnelheid lager in de patiëntengroep, terwijl andere spatiele en temporele gangparameters hetzelfde waren. De patiënten met de grootste laesies in de vermis hadden het minst stabiele gangpatroon.

In het tweede deel lag de focus op split-belt adaptatie, een populaire methode om de rol van het cerebellum binnen gangadaptatie te besturen. Eerst bekeken we split-belt adaptatie bij patiënten met focale cerebellaire laesies en gezonde controle proefpersonen. We zagen dat tijdens het split-belt adaptatie experiment de patiënten en controles globaal dezelfde veranderingen in de gangparameters vertoonden. Echter, er was een verschil tussen de groepen in het ‘after-effect’ van de standtijdsymmetry: tijdens de eerste fase van de post-adaptatie periode hadden de patiënten een grotere asymmetrie in de relatieve standtijden dan de controles. De patiënten die met een grotere asymmetrie in de relatieve standtijden wandelden, hadden een grotere kans om laesies te hebben in de vermislobules VI en Crus II. In de laatste studie, keken we naar de rol van lichaamsbesef in de neurale sturing van split-belt wandelen. We zagen dat de proefpersonen die het minst goed een snelheidsverschil konden voelen, tijdens split-belt wandelen aanvankelijk met meer asymmetrische standtijden stapten. Dit sluit aan bij onze algemene visie dat

belastings- en bewegingsinformatie belangrijk zijn bij de neurale sturing van het wandelen.

Samengevat, het cerebellum is belangrijk bij de sturing van dynamische gangstabiliteit. Daarnaast suggereert onze data dat het cerebellum niet primair betrokken is bij cutane relfex modulatie, maar wel een rol zou kunnen spelen bij het verminderen van zelfgeïnduceerde reflexen. Ons onderzoek toonde dat mild ataxische cerebellaire patiënten geen afwijkingen vertonen tijdens split-belt adaptatie, maar wel verschillen in de post-adaptatie periode. Ten slotte, de waargenomen relaties tussen lichaamsbesef en gangasymmetrie tijdens split-belt wandelen bevestigden het belang van proprioceptieve informatie bij de sturing van het wandelen.

General introduction

Walking is a relatively easy task; we can walk without thinking about how to do it. We think about the items on our shopping list when walking through supermarket aisles, we look for wildlife when we hike and we enjoy the sunset during a summer night's walk along the beach. All of that is possible because we do not need to think about how to move our limbs.

While we are able to walk without thinking about it, from a control perspective, walking is definitely not a simple task. Walking needs to be stable and efficient in a broad range of different conditions, able to handle various perturbations. Insights into the neural control of gait are important to teach us about why specific individuals (patients, elderly) experience difficulties with walking, in general or under specific conditions. With this knowledge rehabilitation and fall prevention strategies can be optimized.

The spinal circuitry has an important role in the neural control of walking in humans (Duysens and Van de Crommert, 1998; Zehr and Stein, 1999). Neuronal networks in the spine generate a motor pattern (alternating activity in flexor and extensor muscle groups); in addition parts of the muscle activation patterns are reflexively induced by afferent input (Dietz, 2002; Duysens and Van de Crommert, 1998; Grillner and Wallén, 1985; Zehr and Stein, 1999). On top of this, multiple supraspinal structures have been suggested to contribute substantially to the neural control of gait (for review: Jahn et al., 2008). An important supraspinal, subcortical structure is the cerebellum. Specific gait deficits in cerebellar patients suggest that the cerebellum is indeed important in the neural control of gait (for review: Ilg

and Timmann, 2013). This is widely supported by observations in animal studies (for review: Armstrong and Marple-Horvat, 1996; Shik and Orlovsky, 1976; Thach et al., 1992; Thach and Bastian, 2004). However, while findings from animal models provide many valuable insights, human (bipedal, plantigrade) gait is different from quadrupedal, digitigrade gait. Human gait is assumed to be more under control of supraspinal structures, due to increased balance demands (Jahn et al., 2008; Morton and Bastian, 2003) and the specific role of the tibialis anterior muscle during heel strike in plantigrade gait (Duysens et al., 2004). Fortunately, over the past few years many studies have specifically addressed the role of the cerebellum in human gait, using more specific patient populations, more precise gait analysis tools and recent brain imaging techniques.

Most studies on gait in cerebellar patients involve patients with degenerative cerebellar ataxias, such as spinocerebellar ataxias and idiopathic cerebellar ataxia (for review: Ilg and Timmann, 2013). Under normal walking conditions, these patients show ataxic gait features such as increased step width, impairments in multi-joint coordination and variability in intra-limb coordination and in global gait parameters (for review: Ilg and Timmann, 2013). Gait behavior in these, often severely ataxic, patients is a complex interaction of several factors such as primary motor deficits (balance control, multi-joint coordination), compensation strategies and inaccurate corrections (Ilg and Timmann, 2013). Therefore, studying severely ataxic gait provides limited insights into the specific role of the cerebellum in the neural control of gait under normal, healthy conditions. Furthermore, because of the diffuse nature of the cerebellar damage in degenerative ataxias, no hard claims can be made about the localization of different functions within the cerebellum from studies with degenerative cerebellar ataxia patients.

Ilg and co-workers (Ilg et al., 2008, 2013) studied patients with focal cerebellar lesions to address the localization of different functions of gait control within the cerebellum. They observed that balance control in walking (assessed through step width and medio-lateral sway) was influenced by other cerebellar sub regions than those involved in adaptation of the intra-limb coordination pattern to walking with added mass at the shanks (Ilg et al., 2008). Step length variability was related to sub regions partly similar to those important for balance control in walking (Ilg et al., 2008, 2013).

So far, in humans the role of the cerebellum has been evaluated mainly during unperturbed, steady state walking. An important next step is to study the role of the cerebellum in the control of gait corrections in reaction to perturbations. Real-life walking is rarely steady state and involves many transient events such as gait initiation, gait termination, turning, obstacle avoidance, visually guided

stepping, uphill/downhill walking, and more. In those conditions, the cerebellum can be expected to be even more involved in the control of gait, because of its function in comparing expected and real sensory signals (for review: Bastian, 2006, 2011).

Objective

In this thesis we evaluated the role of the cerebellum in dynamic gait stability, in cutaneous reflex modulation during gait and in locomotor adaptation, all important features of neural control of gait in non-steady state conditions. The objective of the thesis was to increase the understanding of the roles of the cerebellum in the control of gait corrections in reaction to perturbations, and of the localization of these functions within the cerebellum. The thesis consists of two parts. In Part I, we focused on the relationship between gait stability and cutaneous reflex modulation and the role of the cerebellum in these features. In Part II, we focused on locomotor adaptation, using a split-belt paradigm.

Cerebellar anatomy

Many studies have attempted to understand the gross morphologic organization of the cerebellum, and the fact that these studies are high in number reflects the difficulty of the task (Schmahmann et al., 1999). Longitudinally, the best-established terminology divides the cerebellum into three regions (on each side): the vermis (medial cerebellum), the paravermis (intermediate cerebellum) and the hemisphere (lateral cerebellum) (Fig. 1; Apps and Hawkes, 2009). The vermis is associated with the fastigial nuclei, the paravermis with the interposed nuclei and the hemispheres with the dentate nuclei (Fig. 2; Apps and Garwicz, 2005).

Terminology used to refer to the transversal lobules of the cerebellum has been inconsistent for long (for overview: Table 2 in Schmahmann et al., 1999), but was standardized by the introduction of Magnetic Resonance Imaging (MRI) atlases by Diedrichsen et al. (2009) and Schmahmann et al. (1999). In this thesis, we use the standardized terminology consistent with the latter probabilistic atlas of the human cerebellum (Table 1; Fig. 2; Diedrichsen et al., 2009; Schlerf et al., 2014). Note that there is no true vermis in the anterior lobe (Schmahmann et al., 1999). In addition, paravermal zones were classified by an intermediate/lateral hemisphere ratio of 1 : 3 (Fig. 1; Timmann, D: personal communication; Luft et al., 1998).

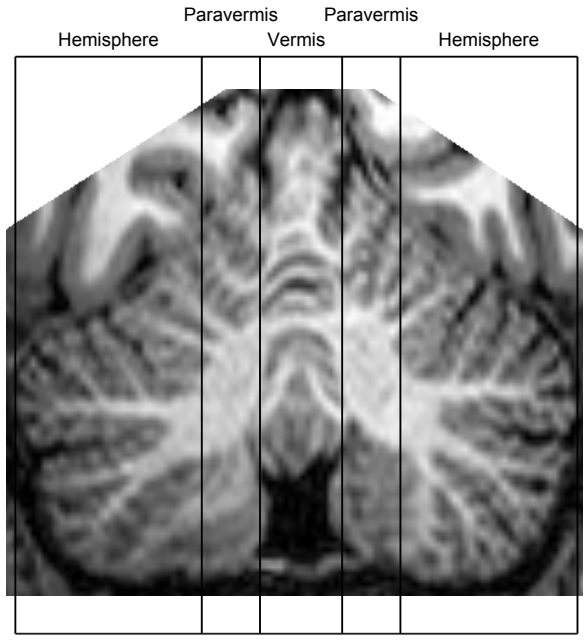


Figure 1: ***Longitudinal zones of the cerebellum.*** *Figure adapted from Luft et al. (1998).*

Table 1: ***Terminology of transversal cerebellar lobules.*** *The first column gives the terminology we used in this thesis. Consensus was defined by Schmahmann et al. (1999), based on a literature review covering 29 publications, including the Nomina Anatomica.*

Lobule	Consensus	Lobe
I II	Lingula	Anterior Lobe
III	Centralis	
IV V	Culmen	
VI	Declive	Superior Posterior Lobe
Crus I	Folium	
Crus II	Tuber	
VIIb	Caudal aspect of Tuber	
VIIIa	Pyramis	Inferior Posterior Lobe
VIIIb		
IX	Uvula	
X	Nodulus	Flocculonodular Lobe

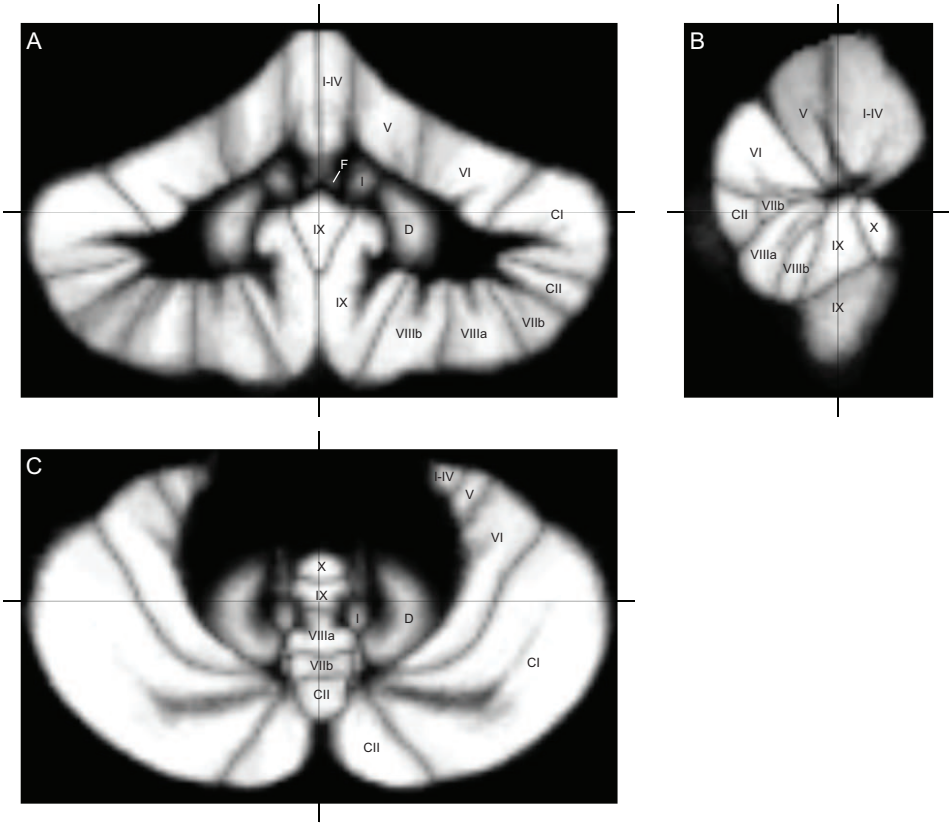


Figure 2: ***Lobules and nuclei of the cerebellum.*** ***A)*** *Coronal slice from probabilistic atlas of the cerebellum (Diedrichsen et al., 2009, 2011); terminology according Diedrichsen et al. (2009). Lobules I-X are represented by their number; F = fastigial nucleus; I = interposed nuclei; D = dentate nucleus. Vertical lines outside the panel indicate the location of the sagital slice; horizontal lines outside the panel indicate location of the axial slice.* ***B)*** *Sagittal slice.* ***C)*** *Axial (transverse) slice.*

Cerebellar function and localization

Functional localization within the cerebellum has a history of more than a century (Manni and Petrosini, 2004). Sensorimotor functions have been getting most attention, but it is now evident that the cerebellum is also critical in the regulation of cognition and emotion (for review: e.g. Koziol et al., 2014; Stoodley and Schmahmann, 2010). Lobules I-VI, together with parts of lobule VIII and the interposed nuclei constitute the sensorimotor cerebellum. Here, the lobules I-IV and VIII have most often been related to the lower limb (Buckner et al., 2011; Stoodley and Schmahmann, 2010), where motor activation is linked to lobules VIIIa and VIIIb and somatosensory activation to VIIIb. Lobules CI-VIIb and parts of lobule VI, together with the ventral part of the dentate nucleus, constitute the anatomical substrate of the cognitive cerebellum. The fastigial nucleus and the posterior vermis are related to limbic functions as panic, sadness, autonomic processing and pain perception. Lobule X is essential in the vestibular system (for meta-analysis: Stoodley and Schmahmann, 2009; for review: Stoodley and Schmahmann, 2010).

For a better understanding of the diverse cerebellar functions it is important to consider the structural and functional connections of the cerebellum. The cerebellum receives input through the inferior and middle cerebellar peduncles. The inferior cerebellar peduncle conveys information from the spinal cord, the vestibular nucleus and the inferior olive (see below). Over the middle cerebellar peduncle, the mossy fibers carry information from the pontine nuclei. The pontine nuclei relay information from the cerebral neocortex. In macaques, dense projections to the pontine nuclei emerge from the cortical motor areas (primary motor cortex, premotor cortex), but in addition the prefrontal cortex and posterior cerebral hemispheres are linked to the cerebellar circuitry through (different) pontine nuclei. Interestingly, in humans the largest proportion of the cortico-pontine fibers comes from the prefrontal cortex, not from the motor areas (for review: Ramnani, 2006). This supports the idea that in humans the cerebellum has important non motor functions as well. In relation to the cerebellar motor functions of interest for this thesis, however, it is important to know that the discussed cortico-pontine-cerebellar pathway is thought to provide an important part of the information used for motor learning in the cerebellum (long-term depression, see below).

Another part of the information important for motor learning comes from the inferior olive over the inferior cerebellar peduncle. The inferior olive is thought to be involved in integrating signals from muscle proprioceptors, specifically those resulting in error signals (sensory consequences resulting from unexpected external

perturbations). Within the concept of long-term depression (for review: Ito, 2002), the axons from the inferior olive, called climbing fibers, carry error signals to the Purkinje cells. Simultaneously, the Purkinje cells receive information from the mossy fibers, via the parallel fibers (axons of the granule cells). After simultaneous stimulation of both climbing and parallel fibers, postsynaptic responses to parallel fiber stimulation are smaller for at least an hour. Error learning through long-term depression has often been suggested to be the basis of the cerebellum's capacity to form and update internal models (e.g. Ito, 2008), but the evidence to support the theoretical role of long-term depression in motor learning is still inconclusive (Ito et al., 2014).

A specific motor learning process for which the cerebellum has been suggested to be important is motor adaptation. There are multiple observations of reduced motor adaptation in cerebellar patients, both in the upper limbs (Burciu et al., 2014; Smith and Shadmehr, 2005; Tseng et al., 2007) and in locomotor paradigms (Ilg et al., 2008; Morton and Bastian, 2006). To further address the possible role of long-term depression of Purkinje cells in locomotor adaptation, Jayaram et al. (2011) used transcranial magnetic stimulation to assess depression of cerebellar excitability due to split-belt adaptation (see below). They observed that after a split-belt adaptation trial, the inhibitory tone that the cerebellum normally exerts over the primary motor cortex was reduced. The amount of inhibition reduction was correlated to the change in step length symmetry during the split-belt condition (adaptation). Furthermore, after other complex walking conditions where no adaptation occurred, this inhibition reduction was not present. Finally, changes in excitability of the primary motor cortex itself were not specific to adaptation, but rather to task complexity. Together these findings support the theory that adaptive learning is mediated, at least in part, by long-term depression in Purkinje cells (Jayaram et al., 2011).

Observations of impaired adaptation of specific gait parameters in cerebellar patients, combined with adequate adjustments in other gait parameters (for details: chapter 4), supported the notion that the cerebellum plays a more important role in feedforward control than in feedback control (for review: Bastian, 2006). The cerebellum functioning as sensimotor predictor fits well within popular motor control theories. Such an internal model function, however, is not easily distinguished from another often suggested function for the cerebellum: internal clock (for review: Ivry and Spencer, 2004). For example, reaching to a target requires precise timing of muscle activity to decelerate the arm. Overshooting, which is often observed in cerebellar patients, could therefore result from either a defunct timing mechanism or an impaired internal model (Bastian, 2011). Interestingly, the neural clock function of the cerebellum has been suggested to involve the same elements as

the error signal function: the inferior olive, climbing fibers and Purkinje cells (for review: Xu et al., 2006).

For gait, the cerebellar input from the spinal cord, through the spinocerebellar tracts, is of specific interest. The dorsal spinocerebellar tract carries information from muscle spindles and Golgi tendon organs and synapse with neurons of Clarke's nucleus. Axon fibers of those neurons generally pass through the inferior cerebellar peduncle into the cerebellum. The ventral spinocerebellar tract generally enters the cerebellum through the superior cerebellar peduncle. It has been commonly accepted that during gait the dorsal spinocerebellar tract relays sensory information from the legs, while the ventral spinocerebellar tract is less influenced by peripheral inputs and more by circuits generating the gait pattern (spinal central pattern generators). However, recent evidence supports the notion that the ventral and dorsal spinocerebellar tracts are much more similar than previously thought (for review: Stecina et al., 2013). It appears that the majority of dorsal spinocerebellar tract cells can be driven by spinal central pattern generators, and as such, these neurons could contribute to distinguish sensory consequences of internally generated actions from sensory inputs resulting from external perturbations.

Localization of cerebellar functions related to walking has furthermore been addressed using different imaging techniques, either by measuring brain activity during (imagined) walking, or by lesion symptom mapping (see above). la Fougère et al. (2010) compared brain activity during real walking (assessed through positron emission tomography) with imagined walking (assessed through functional MRI) and observed brain activity in vermal regions around the anterior lobe and lobule VIII. Ilg et al. (2008) applied lesion symptom mapping and observed that dynamic balance control (step width & medio-lateral sway) was influenced by the fastigial nuclei (and to a lesser degree the interposed nuclei) and the inferior posterior lobe of vermis. Adaptation of the intra-limb coordination pattern to walking with added mass at the shanks was related to lesions in the interposed and the adjacent dentate nuclei (Ilg et al., 2008). In a study of the working memory interaction with motor tasks, step length variability was observed to be related to lesions in the dorsal dentate nuclei, extending into the interposed nuclei, and to lesions in vermal lobules VIIIa, VIIIb and IX and paravermal lobules V, VI and IX (Ilg et al., 2013). Step length variability during tandem walking was related to lesions in the dentate nucleus and lobules V, VI, VIIIa, VIIIb and IX, but these lesions were more lateral (Ilg et al., 2013).

Cerebellar patients

The studies were performed with both healthy controls and patients with focal lesions after cerebellar tumor resection. All participants were (young) adults and patients were at least 4 years post-operation to ensure stable chronic lesion conditions. All patients had experienced a tumor at young age (< 15 years old). Cerebellar tumors form the majority of childhood brain tumors (McKean-Cowdin et al., 2013). Pilocytic astrocytomas are most common (26% of all cases; age adjusted incidence rate: 0.86), followed by medulloblastomas (22% of all cases; age adjusted incidence rate: 0.71) (McKean-Cowdin et al., 2013). Eleven patients in our experimental group had experienced a pilocytic astrocytoma; five patients had experienced a medulloblastoma. Furthermore, two patients had focal lesions due to resection of rarer tumor types. One patient had hemangioblastoma, an uncommon vascular tumor of the central nervous system, mostly occurring in subcortical structures as the cerebellum, brainstem and spinal cord (Bamps et al., 2013). Furthermore, one patient with Lhermitte Duclos Disease was included, a very rare condition of which less than 100 cases had been reported in literature up to 2007 (Kumar et al., 2007).

The different studies in this thesis were performed with different subsamples of the same sample of 18 patients with focal cerebellar lesions. We aimed to include more patients, but not many patients met our inclusion criteria (age > 18 yrs, focal lesion after tumor resection, > 2 yrs postop, able to walk independently). Some patients participated in all patient studies, others in only one experiment (Table 2). This work was performed within a larger project and not all experiments are reported in this thesis. An initial cohort of 13 patients was recruited and performed experiments for the cutaneous reflex modulation study (chapter 2), the gait stability (chapter 3) and the split-belt adaptation study (chapter 4). These patients first performed the cutaneous reflex modulation experiment. After a short break they performed the split-belt adaptation protocol. The baseline trial of the split-belt adaptation protocol was also used for the gait stability study. Due to repeated technical issues with the treadmill some of these patients could not perform all the individual trials (Table 2).

As soon as the protocol for an additional fifth study (C-Mill, outside the scope of this thesis) was finalized, an additional cohort of 5 patients was recruited. These patients performed experiments for the gait stability (chapter 3), the split-belt adaptation study (chapter 4) and the additional C-Mill study. One of these patients could not perform the split-belt adaptation and C-mill experiments without holding the hand railing and was excluded from these studies. All patients of the first

Table 2: ***Overview of which patients participated in which experiments***

#	Diagnosis	Lesion volume (cm^3)	ICARS /100	RT	CT	Cutaneous Reflexes Chapter 2	Gait Stability Chapter 3	Split-belt Adaptation Chapter 4	Perception Threshold Chapter 5	Cmill
1	LDD	58.0	3			Y	Y	Y	Y	Y
2	Pilocytic Astrocytoma	8.2	3			Y	Y	Y	Y	Y
3	Medulloblastoma	22.0	13	Y		Y	Y	Y	Y	Y
4	Pilocytic Astrocytoma	1.7	6			Y	Y	Y	Y	Y
5	Medulloblastoma	22.6	2	Y	Y	Y	Y	Y	Y	Y
6	Medulloblastoma	6.3	19	Y	Y	Y	Y	Y	Y	Y
7	Medulloblastoma	5.4	5	Y	Y	Y	Y	Y	Y	Y
8	Astrocytoma grade II	7.1	0	Y		Y	Y	Y		
9	Hemangioblastoma	no MRI	6			Y	Y	Y		
10	Pilocytic Astrocytoma	58.4	5	Y		Y	Y	Y		
11	Astrocytoma grade III	8.6	11	Y		Y	Y			
12	Medulloblastoma	14.2	17	Y	Y		Y	Y		
13	Pilocytic Astrocytoma	47.3	3				Y			
14	Pilocytic Astrocytoma	4.5	1				Y	Y	Y	Y
15	Astrocytoma grade II	2.0	1				Y	Y		Y
16	Pilocytic Astrocytoma	36.3	2				Y	Y		Y
17	Pilocytic Astrocytoma	15.7	7				Y	Y		Y
18	Pilocytic Astrocytoma	20.2	20	Y			Y			
Total				9	4	11	18	15	8	11

cohort who performed the split-belt adaptation experiment ($n = 11$) were invited to return to the laboratory to perform the perception threshold paradigm (chapter 5) and the additional C-Mill study; seven of them participated. Finally, one of the patients of the second cohort was willing to return to the laboratory to perform the perception threshold paradigm (chapter 5).

Lesion symptom mapping in the cerebellum is preferably performed with patients with pilocytic astrocytomas or hemangioblastomas (Timmann et al., 2009). Less useful conditions involve accompanying hydrocephalus or additional radiation and therapy chemotherapy, due to their (long-term) effects on brain function (Timmann et al., 2009). Furthermore, it should be noted that findings in these patients may be confounded by the flexibility of the maturing central nervous system and by potential early compensation taking place during the slow progression of the tumor (Timmann et al., 2009). In lesion symptom mapping, patients are commonly grouped either by lesion or by behavior (Bates et al., 2003). In the lesion based approach, subgroups of patients with and without lesions are composed and behavioral outcomes are compared between subgroups. In the behavior based approach, subgroups of patients with unaffected behavior (values similar to healthy control values) and patients with affected behavior are identified. For this classification, commonly a cut-off threshold is applied, based on the behavioral data of the healthy controls, which is not always straightforward (Hoogkamer and Meyns, 2014). Then, lesions from patients with affected behavior are superimposed and compared to lesion

overlaps of unimpaired patients; this is done on a voxel-by-voxel basis (Bates et al., 2003).

General experimental paradigm

For all walking experiments participants walked on an instrumented dual-belt treadmill (custom built by Forcelink, Culemborg, The Netherlands). Three-dimensional ground reaction forces were recorded at 1,000 samples/s. In addition to the kinetic data we collected muscle activity data (electromyography; EMG) during the cutaneous reflex experiments and three-dimensional kinematics during the gait stability and split-belt adaptation experiments. Kinematics were recorded at 100 samples/s (Vicon Nexus, Oxford Metrics, Oxford, UK) using markers at the pelvis and ankles.

Cutaneous reflex modulation and suppression

In walking humans, non-noxious electrical stimulation of cutaneous nerves around the ankle results in reflex activity in lower limb muscles (Duysens et al., 1990; Yang and Stein, 1990). The magnitude of these cutaneous reflexes is not just a function of background activity in the muscle at the moment of the reflex, but differs between different phases of the movement cycle (Duysens et al., 1990; Tax et al., 1995; Yang and Stein, 1990; Zehr et al., 1997). This phase-dependent modulation has often been suggested to have a role in maintaining stable locomotion (Haridas et al., 2005, 2008; Tax et al., 1995; Zehr and Stein, 1999). We used a backward walking paradigm in healthy controls to decrease gait stability (see review on backward walking: Hoogkamer et al., 2014c) and assessed cutaneous reflexes in both legs in this condition. In addition we evaluated phase-dependent reflex modulation and suppression of reflex amplitude after self-induced stimuli in cerebellar patients.

While participants were walking, an electrical stimulus was repeatedly applied at the sural nerve near the ankle of the right leg. We recorded EMG in the biceps femoris, tibialis anterior and gastrocnemius medialis muscles of both legs at 1,000 samples/s. In the first experiment (backward walking) and in the 'externally-triggered' condition of the second experiment, the stimuli were automatically triggered by the software, based on heel strike detection (custom-written in MATLAB). In the 'self-induced' condition of the second experiment the stimuli were manually triggered by the participants. For all conditions the target was to

collect reflex activity to 10 stimuli in 16 equidistantly distributed phases in the gait cycle. Per phase we quantified the reflex responses by calculating the mean reflex activity and subtracting mean background activity (during same time window of the preceding step). Reflex activity during different phases of the gait cycle was compared between conditions and groups.

Gait stability

Gait can be considered to be stable if it does not lead to falls in spite of perturbations (Bruijn et al., 2013; Calandre et al., 2005). Such perturbations can arise from external sources (e.g. surface friction and/or uneven surfaces) but also from internal sources (e.g. neuromuscular) (Bruijn et al., 2013). In patient studies the stride-to-stride variability of stride time, step length or step width are often used to infer gait stability (for review: Bruijn et al., 2013). However, from a biomechanical perspective, increased variability itself does not necessarily imply decreased stability. This is best illustrated with an example from the recent review on gait stability measures by Bruijn et al. (2013): "... consider two subjects with identical anthropometrics, walking with identical trunk motions and equal step width variability, but different step widths. The subject with the smaller step width is more likely to tip over, and, thus, variability alone is not sufficient to describe stability in this case. Both subjects have the same constraints (i.e. keeping the centre of mass (or, rather, the extrapolated centre of mass, ...) within the base of support), but have a different control strategy (i.e. one of them walks with a larger step width), and, thus, variability does not in this case index gait stability." As such, the relationship between variability and stability is not straightforward; one should know both the constraints and the control strategy of a system.

Alternatively, for other gait stability measures, derived from biomechanics or from dynamical systems theory, the relation with the probability of falling is more straightforward given their definition. A popular concept derived from biomechanics is the 'extrapolated center of mass' concept (Hof et al., 2005). Within this concept, the 'margin of stability' quantifies how close an inverted pendulum model of the participant would be from falling. The concept extends the condition for static stability (the vertical projection of the center of mass should be within the base of support) to dynamic situations. Assuming the body to be an inverted pendulum, a linear function of the center of mass velocity is added to the center of mass (together called the 'extrapolated center of mass'). For dynamic stability this 'extrapolated center of mass' should be within the base of support (Hof et al., 2005). The distance between those two is referred to as 'margin of stability' and a greater

margin is associated with more stable gait. However, to what extent the margin of stability adequately reflects experimentally induced changes in stability, is still under debate (Bruijn et al., 2013).

An increasingly popular measure to quantify gait stability, derived from dynamical systems theory, is the maximum Lyaponov exponent. It is calculated from a steady-state walking pattern without any additional external perturbations and quantifies the system's ability to recover from small perturbations, represented by the natural stride-to-stride variations that occur during locomotion (Dingwell et al., 2000). Not only is, given this definition, the relation of the maximum Lyapunov exponent (λ) with the probability of falling plausible, its validity has been supported by modeling (e.g. Bruijn et al., 2012b; Roos and Dingwell, 2011), experimental (e.g. Chang et al., 2010; Sloot et al., 2011) and observational studies (e.g. Toebes et al., 2012; van Schooten et al., 2014), specifically for the short-term maximum Lyapunov exponent (λ_S). Among current gait stability measures the validity of λ_S is best supported across all validity levels (Bruijn et al., 2013).

For the study on dynamic gait stability in patients with focal cerebellar lesions and healthy controls, we calculated the short-term maximum Lyapunov exponent from the medio-lateral displacement of the posterior pelvis markers, following Bruijn's protocol (Bruijn et al., 2009). The first derivative of the medio-lateral displacement was used to estimate the stability, to overcome non-stationarities (Bruijn et al., 2009; Dingwell and Marin, 2006). Time series of 150 strides were time-normalized to on average 100 samples per stride. From the time-normalized time-series and its time-delayed copies, state spaces were reconstructed:

$$S(t) = [v(t), v(t+\tau), v(t+2\tau), v(t+3\tau), v(t+4\tau)]$$

with $S(t)$ representing the 5 dimensional state vector, $v(t)$ the original 1 dimensional medio-lateral velocity data and τ the selected embedding delay (Fig. 3). An embedding dimension of 5 was used because this proved to be sufficient to capture the dynamics of human gait (Dingwell and Cusumano, 2000) and based on global false nearest neighbor analysis (Bruijn et al., 2009; Kennel et al., 1992). A standard embedding delay of 10 samples was used (Bruijn et al., 2009; England and Granata, 2007), as a fixed delay has been observed to result in the best within- and between-session intra class correlatin coefficients and the smallest detectable differences (van Schooten et al., 2013). Then, we tracked the Euclidean distance between each data point in state space and its nearest neighbor over time. A divergence curve was constructed by taking the mean of the log of all these time-distance curves. The short-term maximum Lyapunov exponent is the slope of this divergence curve over $0 - 0.5$ strides ($0 - 50$ samples). Higher values for the maximum Lyapunov

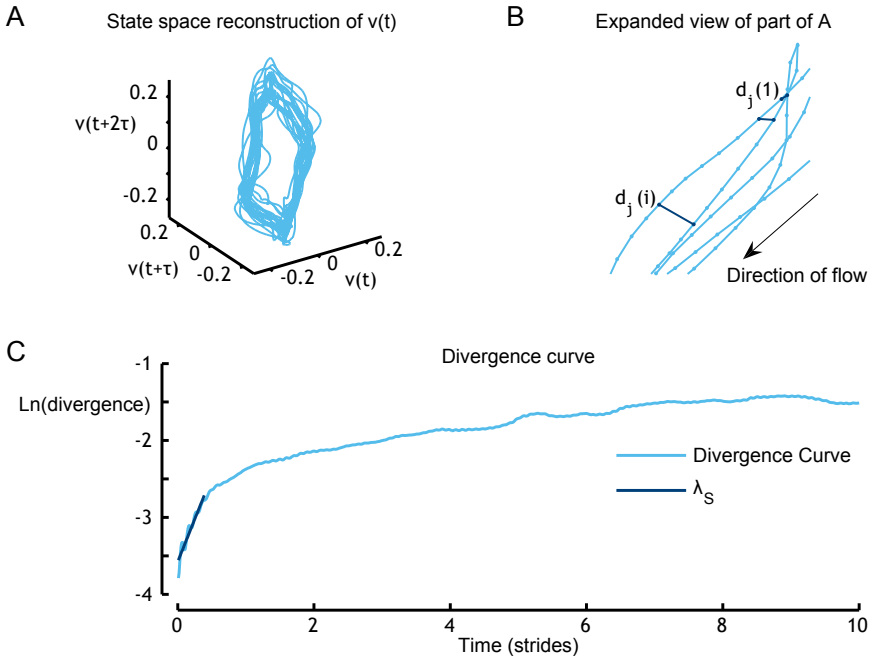


Figure 3: ***Calculation of the maximum Lyapunov exponent.*** ***A)*** *A three-dimensional state space reconstruction of $v(t)$; in practice a five-dimensional reconstruction was applied.* ***B)*** *Close-up view of part of the state space reconstruction; for each point, the nearest neighbor was calculated, and divergence of these points was calculated.* ***C)*** *Average logarithmic rate of divergence, from which the short-term maximum Lyapunov exponent (λ_S) was calculated as the slope of the curve at $0 - 0.5$ strides. Figure adapted from Bruijn et al. (2013).*

exponent imply less local stability.

Split-belt adaptation

To study locomotor adaptation, we used a split-belt paradigm. Split-belt walking can be studied with a dual-belt treadmill, consisting of two parallel belts, one for each leg, which can run at different velocities. Split-belt walking requires a change in inter-limb coordination and the different biomechanical constraints

result in different muscle activation patterns (Dietz et al., 1994). Proprioception and afferent input have an important role in controlling split-belt gait (Dietz et al., 1994; Jensen et al., 1998; Prokop et al., 1995; Zijlstra and Dietz, 1995). During split-belt walking, intra-limb gait parameters such as limb excursion and relative stance time are quickly adjusted (Prokop et al., 1995; Reisman et al., 2005). Inter-limb gait parameters (directly depending on coordination between legs) such as step length and relative double stance time are slowly adapted and stored as new patterns (as identified by the presence of after-effects) (Reisman et al., 2005). This adaptation is commonly assessed during a classic split-belt adaptation paradigm, where participants walk with one of the belts running at twice (or thrice) the speed of the other, for an extended period of time (5 to 15 minutes) (for review: Torres-Oviedo et al., 2011). Initially, participants walk with asymmetric step lengths, taking longer steps with the leg on the slow belt. Over time they adapt their gait pattern towards more symmetric step lengths. This adaptation process results in after-effects when belts are returned to equal speeds. In this post-adaptation condition healthy participants again initially walk with asymmetric step lengths, but now taking longer steps with the leg that has been on the fast belt during the split-belt condition. Within a few minutes these after-effects disappear and step lengths return to baseline (symmetric) values. Patients with severe degenerative cerebellar disease usually do not display these typical features of split-belt adaptation (Morton and Bastian, 2006). We aimed to address the localization of this cerebellar involvement in split-belt adaptation, by evaluating split-belt adaptation in patients with stable focal lesions after cerebellar tumor resection.

The calculation of spatial gait parameters during split-belt walking is not straightforward as we argue in this thesis (Hoogkamer et al., 2014b, see Appendix A of this thesis). Unlike normal treadmill gait, in split-belt gait, temporal parameters and belt speeds cannot be used easily to calculate spatial parameters, as both belts run at different speeds. Thus, spatial parameters need to be calculated from feet positions directly. For step length, this is done by taking the anterior-posterior distance between the ankle marker of each leg at initial contact of the leading leg. Fast step length refers to the step length measured at initial contact of the fast leg (leg which makes contact with the fast moving belt); slow step length refers to step length measured at slow leg initial contact (Reisman et al., 2005) (Fig. 4). It is important to calculate these distances at initial contact, as the feet will move closer together (or apart) during double support, since the belts move at different speeds (Fig. 4).

For stride length, the situation is more complex, since stride length includes both left and right steps. Logically, one would define stride length as the sum of left and

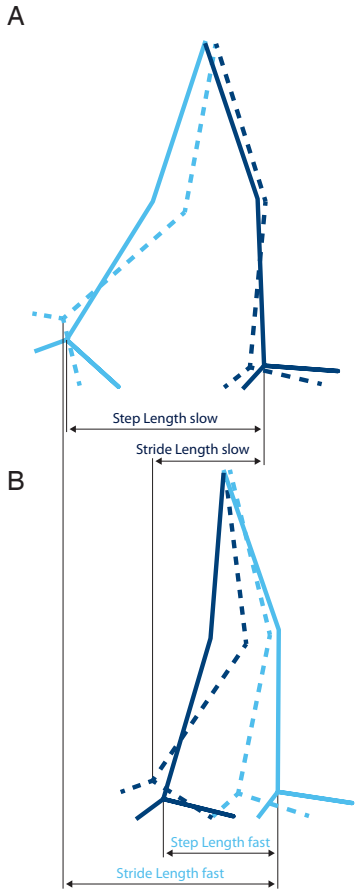


Figure 4: ***Definitions of spatial gait parameters for split-belt gait. A)*** *Side view during initial contact of the slow leg (full lines) and during lift-off of the fast leg (dashed lines).* ***B)*** *Side view during initial contact of the fast leg (full lines) and during lift-off of the slow leg (dashed lines). A 'fast step' occurs when moving from the dashed lines in panel A to the full lines in panel B, while a 'slow step' occurs when moving from the dashed lines in panel B to the full lines in panel A. The interval between initial contact of the leading leg (full lines) and the lift-off of the trailing leg (dashed lines) consists of the double support phase. Step length is calculated by taking the anterior- posterior distance between the ankle marker of each leg at initial contact of the leading leg; fast step length refers to the step length measured at fast leg initial contact and slow step length refers to step length measured at slow leg initial contact. Stride length is calculated as the distance traveled by the ankle marker in the anterior-posterior direction from initial contact to lift-off of one limb. Figure from Hoogkamer et al. (2014b, see Appendix A of this thesis)*

right step lengths, similar to normal gait. However, in their influential paper on split-belt adaptation Reisman et al. (2005) introduced a modified version of stride length, which is calculated as the distance travelled by the ankle marker in the anterior-posterior direction from initial contact to lift-off of one limb (Fig. 4). The advantage of this method is that it allows assessing asymmetry in 'stride lengths' between fast and slow legs. However, this type of stride length is not comparable to 'normal' stride length, which can never be asymmetrical on a treadmill.

Instead, this definition of 'stride length' is closer to a measure of limb excursion, or distance travelled during stance. While the group who introduced this measure (Reisman et al., 2005) moved away from the use of stride length and focused on other spatial parameters based on limb excursion (Malone et al., 2012), we, and other groups, initially adopted their definition of stride length to be consistent (Bruijn et al., 2012c; Nanhoe-Mahabier et al., 2013). However, we have to acknowledge that stride length is not the most appropriate name for this gait parameter, since in any other condition 'stride' refers to a complete gait cycle. Alternatively, we argue that in future research this measure is best referred to as 'limb excursion'; this to avoid any confusion with 'true' stride length.

Our split-belt paradigm consisted of ten minutes of walking with one belt at 1.0 m/s and the other belt at 0.5 m/s, followed by five minutes with both belts at 1.0 m/s (Bruijn et al., 2012c). We evaluated adaptation and after-effects in step length symmetry (Choi et al., 2009):

$$\text{Step Length Symmetry} = \frac{step\ length_{fast} - step\ length_{slow}}{step\ length_{fast} + step\ length_{slow}}$$

Similarly, symmetry values were calculated for the relative double stance time, limb excursion and relative stance time of each leg.

Perception of locomotor asymmetry

Furthermore, we aimed to test two opposing hypotheses about the control of split-belt walking. Therefore we explored relationships between gait parameters during split-belt walking on one hand and how well participants were able to perceive a difference in belt speeds on the other hand. To assess the latter we used a perception threshold paradigm that was recently introduced by Lauzière et al. (2014b). In general their protocol was as follows: initially the belts ran at an equal speed, and then one of the belts speeds was increased by a step of 0.01 m/s every 5 seconds, until the participants reported that they perceived themselves as walking asymmetrically. In our protocol, only trials were performed where one of the belt

speeds was increased. We applied an acceleration of 0.00278 m/s (0.01 km/h) each second.

Outline

This thesis on the role of the cerebellum in the neural control of gait corrections consists of two parts. The first part (chapters 1 to 3) focuses on dynamic gait stability and cutaneous reflex modulation. The second part (chapters 4 and 5) focuses on locomotor adaptation, using a split-belt paradigm.

In the first part, 'Gait stability, cutaneous reflex modulation and the cerebellum' we focused on the relationship between gait stability and cutaneous reflex modulation and the role of the cerebellum in these features.

In **chapter 1** we aimed to find a relationship between cutaneous reflex modulation and dynamic gait stability. We used a backward walking paradigm in healthy controls to decrease gait stability and we assessed cutaneous reflexes in both legs in this condition. Observations from earlier studies suggested an important role for cutaneous reflexes in contralateral leg muscles in maintaining stability (Haridas et al., 2005, 2008; Lamont and Zehr, 2007; Tax et al., 1995). As backward walking is unusual and more variable than forward walking (Hoogkamer et al., 2014c), we hypothesized that backward walking would be less stable than forward walking and that during backward walking the muscles in the contralateral leg would show pronounced reflex activity during its stance phase. Furthermore, we expected a correlation between reflexes in the contralateral leg and gait stability during backward walking.

In **chapter 2** we evaluated cutaneous reflexes during walking in patients with focal cerebellar lesions. Both phase-dependent reflex modulation and suppression of self-induced reflexes were addressed. Although this phase-dependent reflex modulation has been studied extensively (for review: Zehr and Duysens, 2004), precise knowledge about the underlying neural pathways is still incomplete. To some degree this modulation is thought to be established through locomotor pattern generating neural networks located at spinal level (Duysens et al., 2004), but in addition, a recent study in rats suggested a role for the cerebellum in this modulation (Pijpers et al., 2008). We hypothesized that phase-dependent reflex modulation patterns would be less pronounced in patients. Furthermore, we expected the cerebellum to be important in the suppression of reflexes to self-evoked stimuli, a phenomenon previously observed in healthy controls (Baken et al., 2006). Such a role for the cerebellum would be in line with its involvement in the prediction of

sensory consequences of actions (Bastian, 2011). It was hypothesized that cerebellar patients would show less reduction of reflexes when stimuli are self-induced as compared to healthy controls.

In **chapter 3** we evaluated dynamic gait stability in patients with focal cerebellar lesions and in healthy controls. We hypothesized that patients would walk with a less stable gait pattern and with a reduced margin of stability. Specifically, we expected that the short-term maximum Lyapunov exponent would make an important contribution to the description of gait deficits in this mildly ataxic patient group.

In the second part, 'Split-belt adaptation, somatosensory perception and the cerebellum' we approached split-belt adaptation from multiple perspectives.

In **chapter 4** we aimed to address the localization of the cerebellar involvement in split-belt adaptation. Although the mechanisms behind locomotor adaptation are still not fully understood, the cerebellum is thought to have an important role (Hoffland et al., 2014; Ilg et al., 2008; Jayaram et al., 2011, 2012; Morton and Bastian, 2006). For instance, Morton and Bastian (2006) were able to show that severely ataxic patients with degenerative cerebellar disease do not display the typical features of split-belt adaptation. So far, it is still under debate where this involvement is localized within the cerebellum (Ilg et al., 2008; Morton and Bastian, 2006). We hypothesized that patients with focal cerebellar lesions would show several impairments during split-belt adaptation and that these impairments would be most pronounced in patients with lesions in the interposed nuclei.

In **chapter 5** we aimed to test two opposing hypotheses about the control of split-belt walking. The first hypothesis proposes that participants aim to minimize limping during split-belt walking; hence they try to walk with symmetric durations of gait phases (stance symmetry hypothesis). Alternatively, the second hypothesis states that left-right differences in limb excursion are minimized (excursion symmetry hypothesis). This would be in line with the concept that the swing phase is initiated when the hip passes through a particular threshold angle and therefore subjects try to reach that angle on both sides of the body (for review of the evidence: Duysens et al., 2000). To test this we assessed how well participants, both cerebellar patients with focal lesions and healthy controls, could feel differences between belt speeds during split-belt treadmill walking and correlated this to their split-belt adaptation parameters. If the first hypothesis is true, then participants who are better able to perceive differences between belt speeds will walk with more symmetry in stance time than participants who are less able to perceive belt speeds differences. Because stance time is inversely coupled to limb excursion during

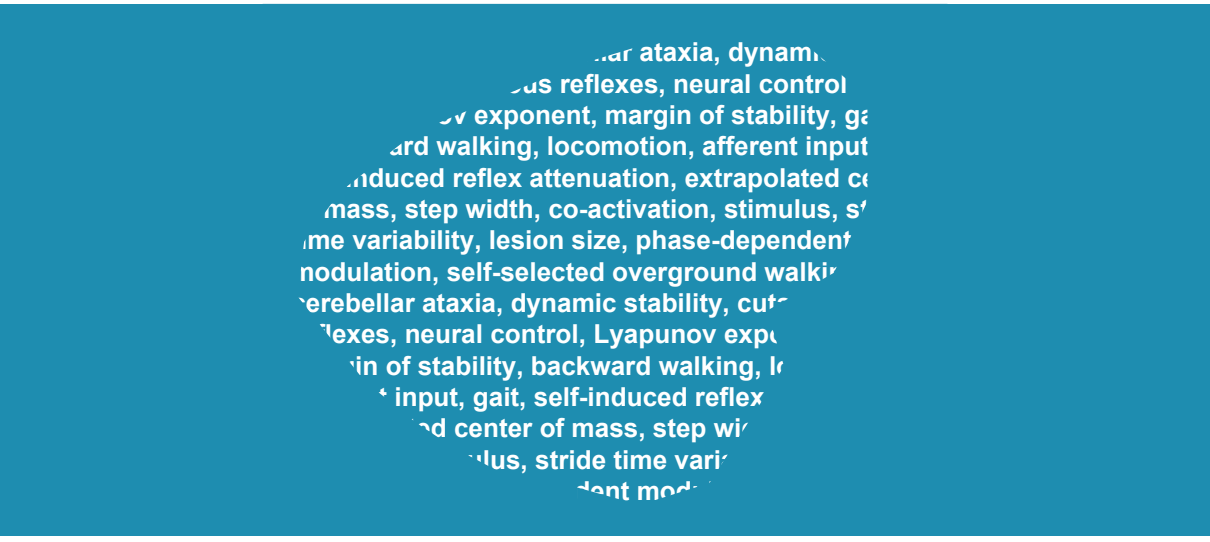
treadmill walking, the participants with best somatosensory perception will walk with the most asymmetric limb excursions. If the second hypothesis is true, the relations between perception and split-belt gait parameters will be the inverse from what is described above.

The thesis ends with a **General Discussion** which summarizes the main findings of both parts and provides general insights based on these. Furthermore, we address limitations and future research directions.

As appendices, we included two additional articles on split-belt gait, a technical note and a commentary, that are relevant for the second part of the thesis. In **Appendix A** we argued that the use of the term 'stride length' in split-belt literature is confusing, as it is importantly different from the standard definition of stride length. We suggested this parameter is actually a measure of limb excursion, should be referred to as limb excursion in future studies. In **Appendix B** we discussed the perception threshold paradigm, introduced by Lauzière et al. (2014b) and addressed which gait parameters are closely related to the difference in belt speeds. Furthermore, we discussed which sensory information the participants could be using to identify gait asymmetry.

Part I

Gait stability, cutaneous reflex modulation and the cerebellum



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Cutaneous reflexes and gait stability in backward walking

Published in Journal of Neurophysiology as:
Hoogkamer W, Massaad F, Jansen K, Bruijn SM, Duysens J. Selective bilateral activation of leg muscles after cutaneous nerve stimulation during backward walking. *J Neurophysiol* 108: 1933-1941, 2012.

Abstract

During human locomotion, cutaneous reflexes have been suggested to function to preserve balance. Specifically, cutaneous reflexes in the contralateral leg's muscles (with respect to the stimulus) were suggested to play an important role in maintaining stability during locomotor tasks where stability is threatened. We used backward walking (BW) as a paradigm to induce unstable gait and analyzed the cutaneous reflex activity in both ipsilateral and contralateral lower limb muscles following stimulation of the sural nerve at different phases of the gait cycle. In BW, the tibialis anterior (TA) reflex activity in the contralateral leg was markedly higher than TA background EMG activity during its stance phase. In addition, in BW a substantial reflex suppression was observed in the ipsilateral biceps femoris during the stance-swing transition in some participants, while for medial gastrocnemius the reflex activity was equal to background activity in both legs.

To test whether the pronounced crossed responses in TA could be related to instability, the responses were correlated with measures of stability (short term maximum Lyapunov exponents and step width). These measures were higher for BW compared to forward walking, indicating that BW is less stable. However, there was no significant correlation between these measures and the amplitude of the crossed TA responses in BW. It is therefore proposed that these crossed responses are related to an attempt to briefly slow down (TA decelerates the center of mass in the single stance period) in the light of unexpected perturbations, such as provided by the sural nerve stimulation.

Keywords

Cutaneous reflexes; human gait; phase-dependent modulation; stability; task dependency

1.1 Introduction

Muscle reflex activity plays an important role in the regulation of stable locomotion (Zehr and Stein, 1999; Zehr and Duysens, 2004). In humans, non-noxious electrical stimulation of cutaneous nerves around the ankle results in reflex activity in both lower limb muscles (Duysens et al., 1990; Yang and Stein, 1990) and upper limb muscles (Calancie et al., 1996; Zehr et al., 2001). This reflex activity is not just a function of background activity in the muscle, but fundamental changes in reflexes can be observed by changing features as the stimulus intensity (Duysens et al., 1992), the stimulus location (Van Wezel et al., 1997; Zehr et al., 1997) or the task performed (Kanda and Sato, 1983; Tax et al., 1995). Furthermore, within a certain task (walking, running, cycling) the reflex amplitude can change between different phases of the movement cycle (Balter and Zehr, 2007; Duysens et al., 1990; Mileva et al., 2004; Tax et al., 1995; Yang and Stein, 1990; Zehr et al., 1997). This phase-dependent modulation is typical in the reflex reversal response of the tibialis anterior (TA) muscle of the stimulated leg, where excitatory responses to tibial nerve stimulation during the early swing phase switch to suppressive responses during the late swing phase (Yang and Stein, 1990). These changes were typically observed in most, but not in all participants.

It has been suggested that this phase-dependent modulation would have functional implications (Duysens et al., 1990). Indeed, Zehr and Stein (1999) reviewed the functional relevance of cutaneous reflexes during human locomotion and concluded that reflexes function 'to preserve balance and ensure a stable walking pattern throughout the step cycle'. More specifically, Tax et al. (1995) studied running and suggested that the reflexes in the contralateral leg (i.e. with respect to the nerve stimulation) help maintaining stable locomotion, while the reflexes in the ipsilateral leg correct for the perturbation. In line with this idea, some studies pointed at important changes in the contralateral reflexes with varying degree of stability (Haridas et al., 2005, 2008; Lamont and Zehr, 2007). For example, when stability was challenged by having participants walk while receiving unpredictable anterior-posterior perturbations at the waist, both with arms crossed and with freely moving arms, cutaneous reflexes in primarily the muscles of the contralateral leg were strongly affected (Haridas et al., 2005). In a follow-up study (Haridas et al., 2008), similar conditions were tested, but this time the perturbations at the waist were exchanged for mechanical perturbations at the dorsum of the ipsilateral foot. Modulated reflexes were observed in both the contralateral medial gastrocnemius (MG) and the ipsilateral TA. The contralateral leg was suggested to assist in maintaining stability, while the ipsilateral leg overcomes the obstacle (Haridas et al., 2008).

Lamont and Zehr (2007) added evidence in the same direction. They collected reflex activity data from muscles in the upper and lower limbs and trunk, evoked by sural nerve stimulation. Inclined walking and stair climbing were used to decrease stability and increase task uncertainty, while the effects of holding a handrail were analyzed. They observed that reflexes were amplified in muscles that were functionally able to restore balance. In line with the findings of Haridas et al. (2005, 2008), it was observed that during unsupported locomotor tasks, the reflexes were most prominent in the contralateral leg muscles, which are best suited to restore balance during these tasks. Taken together, the observations in these studies suggest an important role for cutaneous reflexes in contralateral leg muscles in maintaining stability in locomotor tasks where stability is threatened.

Another paradigm used to induce unstable gait, is backward walking (Schneider and Capaday, 2003). Backward walking is unusual and more variable than forward walking (Choi and Bastian, 2007; Grasso et al., 1998; Hackney and Earhart, 2009; Katsavelis et al., 2010; Kurz et al., 2012; Winter et al., 1989). Schneider and Capaday (2003) observed that the instability related to backward walking resulted in high amplitude H-reflex activity in the soleus muscle. This reflex could be attenuated by procedures reducing the instability. In particular they showed that when participants held the handrail or were trained in walking backwards, the reflex amplitude was reduced. Subsequently, increasing instability after training (by making the participants walk backwards with eyes closed) restored the large reflex activity.

Hence one would expect that backward walking is a good model for unstable gait and one would expect to see changes in crossed reflexes here as well. Cutaneous reflexes have been studied during backward walking but only for ipsilateral reflexes (Duysens et al., 1996). It is hypothesized that the study of cutaneous reflexes in the contralateral limb during backward walking will provide more insight in the functional role of cutaneous reflexes; specifically in addition to earlier reports of the prominent role of contralateral cutaneous reflexes in unstable gait (Haridas et al., 2005, 2008; Lamont and Zehr, 2007).

As backward walking is unusual and more variable than forward walking, we hypothesized that backward walking is less stable than forward walking and that in backward walking the muscles in the contralateral leg would show pronounced reflex activity during its stance phase. To test these hypotheses we applied sural nerve stimulation during backward walking and analyzed both the stability and the middle latency reflex activity in both ipsilateral and contralateral lower limb muscles. To ensure that the present conditions were identical to those reported in a previous study (Duysens et al., 1996), we measured the ipsilateral reflexes as well.

1.2 Methods

1.2.1 Participants and protocol

We recruited thirteen healthy participants (7 males and 6 females, 26.9 ± 3.2 yr, 174.3 ± 8.3 cm, 64.1 ± 6.7 kg; $mean \pm SD$) who had no known history of neurological or motor disorders. All participants were right-handed and right-footed. The experimental protocol was approved by the local ethics committee in accordance with the guidelines of the Declaration of Helsinki, and all participants gave written, informed consent.

Participants walked both forwards (FW) and backwards (BW) on an instrumented dual belt treadmill (ForceLink B.V., Culemborg, The Netherlands), for about 10 minutes per condition. For each condition we set both belt speeds to 1.1 m/s (4.0 km/hr), and the order of FW and BW conditions was randomized across the participants. Participants wore thin socks reaching up to about 1 cm below the malleoli and were instructed to try to walk with the left foot on the left belt and the right foot on the right belt, but to pay little attention to any accidentally misplaced steps.

1.2.2 Experimental set-up

We measured electromyography (EMG) recordings at 1,000 samples/second (ZeroWire, Aurion, Italy) from three muscles in both legs: the tibialis anterior (TA), medial gastrocnemius (GM) and biceps femoris (BF). We selected these muscles, because prominent reflex activity has been observed for these muscles (Baken et al., 2006; Duysens et al., 1990, 1996). A pair of recording surface electrodes (with a diameter of 1 cm) was placed over the muscle belly with an interelectrode distance of 2 cm, parallel with the muscle fibers and close to the motor point. Care was taken not to place the electrodes over the peripheries of the muscles to minimize EMG cross talk. The electrodes were placed after hair shaving, skin abrasion and application of alcohol and ether.

We positioned a bipolar stimulation electrode over the sural nerve, approximately halfway between the lateral malleolis and the Achilles tendon of the right leg. We determined the exact location according to the optimal irradiation of the stimulus. The electrode was then firmly attached to the skin with tape and strapped by an elastic bandage around the ankle. Each stimulus consisted of a 5 pulse (1.0 ms) train at 200 Hz (Grass S88 stimulator connected in series with an SIU5 isolator and

a CCU1 constant current unit, Grass Instruments). We tested both the perception and the irradiation threshold during quiet standing just before the first condition and at the end of the experiment to verify stable stimulus conditions. We set the stimulus intensity to the double of the perception threshold (Duysens et al., 1996).

To enable a reproducible stimulation at 16 equidistantly distributed phases in the gait cycle, we used custom-written Matlab software that timed each stimulus in the right phase based on the instant of heel strike of the right (stimulated) leg. The instant of heel strike was determined by a vertical force (see below) threshold of 10% of bodyweight. Ten stimuli were presented for each of 16 phases of the gait cycle in a random order, such that there were at least one ($mean = 1.03$) complete strides without a stimulus between consecutive stimuli. For each condition 160 stimulated strides were assessed. Before we triggered the stimulation software, subjects walked for 2-5 minutes on the treadmill to acclimatize to (backward) treadmill walking. The instrumented dual belt treadmill enabled collection of the ground reaction forces for each individual leg. The forces were sampled at 1,000 samples/second. The vertical reaction force of the right (stimulated) leg was used for the online distribution of the stimuli over the gait cycle (see above). All forces were stored for offline analyses of gait parameters.

1.2.3 Data analysis

We applied a fourth-order recursive, zero phase-shift, Butterworth low-pass filter with a cutoff frequency of 10 Hz on the raw force data. We determined step width and the instants of heel strike and toe-off, based on the center of pressure (Roerdink et al., 2008). We excluded strides when a foot incidentally was placed on two belts or when two feet were on the same belt. Gait cycle was defined from right heel strike (0%) to the next right heel strike (100%).

To evaluate stability, we estimated the center of mass (CoM) based on the center of pressure and calculated the 'extrapolated center of mass' (XCoM) and the safety margin between the XCoM and the base of support (Hof et al., 2005). Moreover, we calculated short term maximum Lyapunov exponents (λ_S) from the medio-lateral displacement of the estimated CoM following Bruijn's protocol (Bruijn et al., 2009). We included 300 consecutive strides in the evaluation of the stability measures. Note that these strides include both stimulated and unstimulated strides.

The safety margin between the XCoM and the base of support quantifies how close an inverted pendulum model of the walking participant would be from falling. Higher values of this margin are related to more stable gait, but could also result

from a step widening strategy by participants during unstable locomotion (Curtze et al., 2011). Negative values of λ_S indicate that the system (in this case the medio-lateral displacement of the estimated CoM) is locally stable, while positive values indicate local instability. In addition, higher values for λ_S imply less local stability (Bruijn et al., 2009; Dingwell and Cusumano, 2000).

The EMG signals were amplified and high pass filtered (cut-off frequency 3 Hz). After full-wave rectification, we low pass filtered the EMG signals (cut-off frequency 300 Hz). We quantified the reflex responses by calculating the mean of the EMG data over the period in which the responses occurred (Fig. 1.1). A single 40 ms time window (Bastiaanse et al., 2006) was set around the reflexes with middle latencies of about 75-80 ms after the stimulation (Duysens et al., 1993; Haridas et al., 2005; Yang and Stein, 1990) for all 16 phases in each muscle. For each muscle, we estimated the reflex latency based on visual inspection of the subtracted EMG traces (see below). This latency defined the first boundary of the time window (Duysens et al., 1996). For both conditions of walking, we set the 40 ms time window at the same latency. When a muscle showed little or no response, we set the time window based on the reflex latency in other nearby muscles. For each of 16 phases of the gait cycle, we averaged the values of 10 stimulated strides. For each stimulus, we estimated the background EMG activity by calculating the mean EMG over a 40 ms time window, placed at the same relative phase of the preceding unstimulated stride. Again, for each phase of the gait cycle, an average value was calculated over 10 strides. We then calculated the 'net' reflex amplitude by subtracting the background EMG activity from the total reflex EMG amplitude. For inter-muscles and inter-subjects comparison, we normalized the resulting data to the phase with the highest background EMG activity during FW.

1.2.4 Statistical analyses

We present all the results in the text as $mean \pm SD$. We used a traditional level of significance ($\alpha = 0.05$) for all statistical tests, when appropriate this value was corrected for the number of analyses. Paired Student's t-tests were applied to compare stability measures between walking directions. For the statistical analyses we log-transformed EMG values to correct for the positive skewness resulting from the full-wave rectification (e.g. Brown et al., 2011). To compare reflex EMG (stimulated condition) and background EMG (control condition) for all phases of the gait cycle we used a two-way repeated-measures ANOVA (2 conditions $\times$ 16 phases) for both walking directions and all muscles. For a given muscle, when we observed a significant condition main effect or a significant condition $\times$ phase

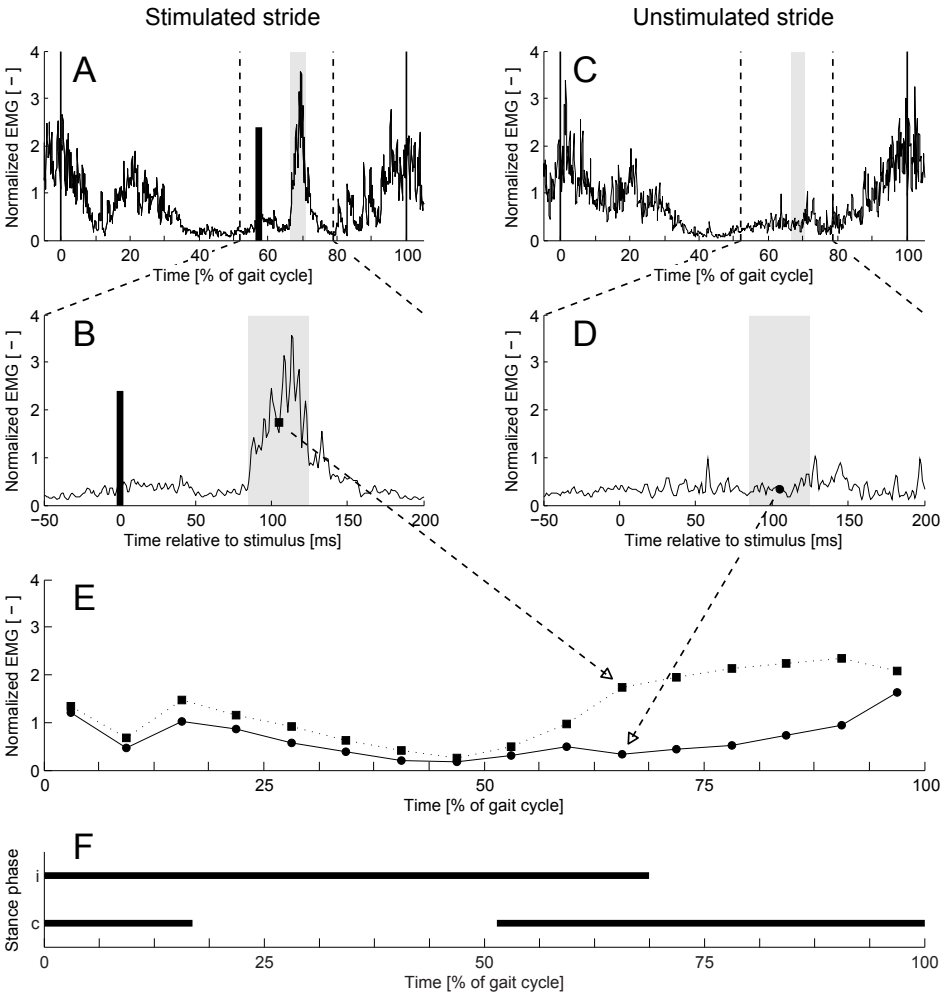


Figure 1.1: ***A)*** *Average normalized reflex EMG of a typical stimulated stride of a single participant. Normalized EMG was averaged for 10 strides with the onset of reflex activity in the same phase of the gait cycle (in this case: phase 11 out of 16). The thick vertical black line indicates the instant of the nerve stimulation.* ***B)*** *Expanded view of the timeframe covering the stimulus and the middle latency response. Reflex EMG (black square) was calculated as the mean value over a 40 ms time window (grey area) starting with an individual determined latency after the stimulus (thick black line).* ***C)*** *and* ***D)*** *Background EMG (black circle) was calculated for the same relative time window of the preceding unstimulated stride.* ***E)*** *This procedure was repeated for all 16 phases of the gait cycle and resulted in a plot of the distribution of the reflex and background EMG over the 16 phases of the gait cycle.* ***F)*** *Duration of the stance phase for the ipsilateral (i) and contralateral (c) leg.*

interaction effect in FW or BW, we performed Tukey's HSD post hoc analysis to determine in which phases reflex and background EMG values were significantly different.

1.3 Results

During backward walking the participants walked with wider steps (0.26 ± 0.03 m) than during forward walking (0.19 ± 0.03 m) ($p < 0.001$), resulting in a higher margin of stability in BW (0.11 ± 0.02 m) compared to FW (0.08 ± 0.01 m) ($p < 0.001$). The short term maximum Lyapunov exponents (λ_S) of the medio-lateral displacement of the estimated CoM during BW was higher (4.98 ± 0.11) than during FW (4.84 ± 0.07) ($p = 0.0025$), indicating that BW is less stable. We had to exclude a single participant (P5) from these analyses, as one of the force sensors in the left belt was not accurately functioning. Determination of the instants of heel strike and toe-off of the stimulated leg (right belt) was not affected, so this participant was included in the reflex analyses.

Typical results for the averaged EMG traces of the contralateral TA (cTA) muscle during backward walking for a single participant (P5) are shown in Fig. 1.1 E. The basic observation was that there were large reflex activations in the cTA during most of contralateral stance. Similar results were obtained for the majority of participants in the population.

1.3.1 Population

For each phase of the gait cycle and each muscle, we calculated the average background EMG and average reflex EMG over all participants during both BW (Fig. 1.2) and FW (Fig. 1.3). To obtain the 'net' reflex activity, background EMG activity was subtracted from reflex EMG activity to obtain 'subtracted EMG responses' (right column of Figs. 1.2 and 1.3).

In Fig. 1.2 it can be seen that the BW data of Fig. 1.1 were confirmed for most participants in the group. On the contralateral side, significant reflex activity was observed in the cTA (phases 10 - 15) and in the BF (cBF) (phases 1, 6, 7, 9 - 12 & 14). At the ipsilateral side, the responses occurred in the TA (iTA) (phases 1 & 16) and BF (iBF) (phases 1 - 3, 10, 11 & 16). The cTA showed significantly enhanced reflex EMG activity during the middle part of the contralateral stance phase. For iBF, reflex EMG activity was below background EMG activity level

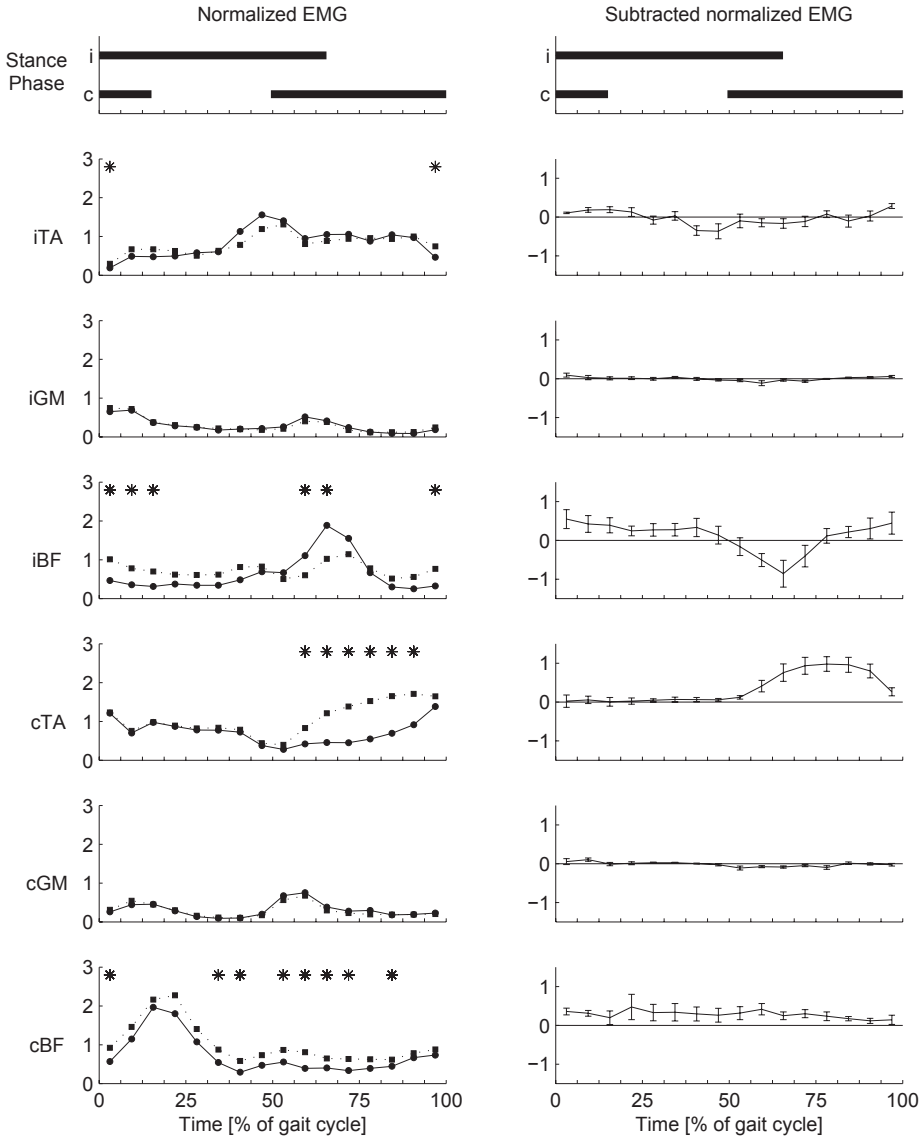


Figure 1.2: ***Background (circle) and reflex (square) EMG values (left column) and subtracted (Reflex - Background) EMG values (right column) for backward walking.*** *EMG values are averaged over all participants. Top panels indicate the duration of the stance phase for the ipsilateral (i) and contralateral (c) leg. Phases when reflex EMG differs from background EMG, are marked by asterisks.*

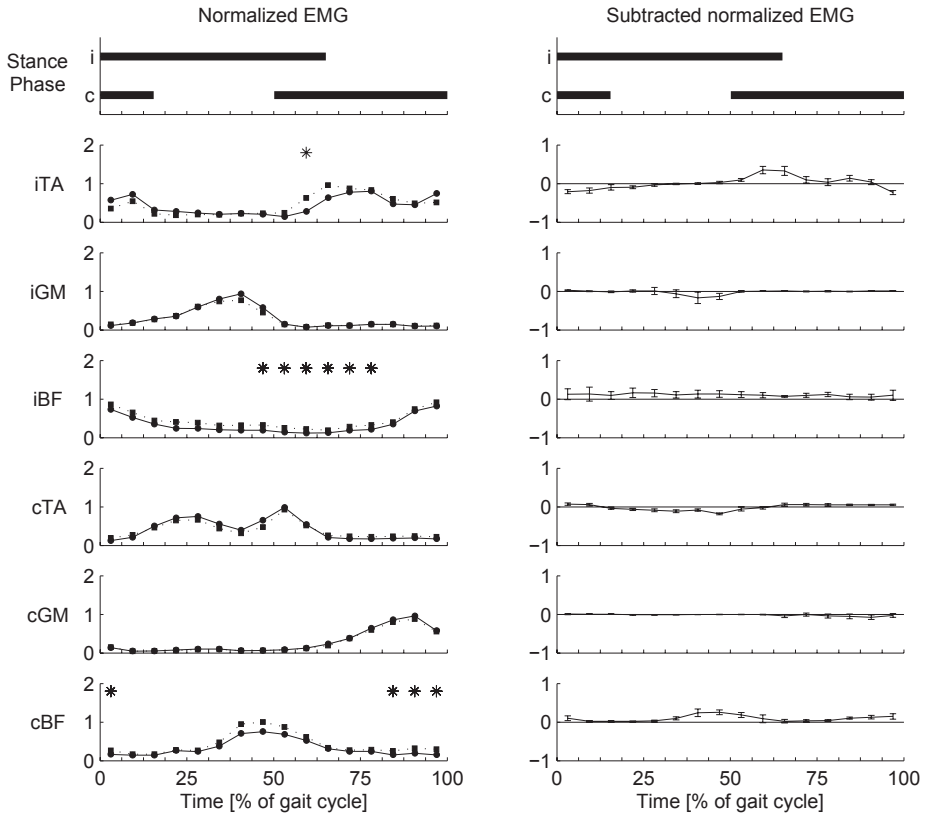


Figure 1.3: ***Background (circle) and reflex (square) EMG values (left column) and subtracted (Reflex - Background) EMG values (right column) for forward walking.*** *EMG values are averaged over all participants. Top panels indicate the duration of the stance phase for the ipsilateral (i) and contralateral (c) leg. Phases when reflex EMG differs from background EMG, are marked by asterisks.*

(suppression) at the end of the stance phase and start of the swing phase of the ipsilateral leg, while reflex EMG activity was enhanced during a period from the end of the swing phase to the start of the stance phase. No significant net reflex activity was observed in the ipsilateral GM (iGM) and contralateral GM (cGM): reflex EMG activity was equal to background EMG activity for all phases of the stride cycle.

For comparison, the same stimuli were also given during FW, to verify that conditions were comparable to previous studies (Baken et al., 2006; Bastiaanse et al., 2006; Duysens et al., 1992, 2010; Lamont and Zehr, 2007; Van Wezel et al., 1997). In FW (Fig. 1.3) significant reflex activity was observed in iTA (phase 10), iBF (phases 8 - 13) and cBF (phases 1 & 14 - 16).

1.3.2 Inter-individual differences

As noted before, there can be substantial inter-individual differences in reflex responses between participants (Baken et al., 2006). This was confirmed in the present study. Here we focus on the cTA and iBF, which displayed phase dependent modulation on a group level (Fig. 1.2). The enhanced reflex activity in the cTA during the contralateral stance phase was observed for 10 out of 13 participants (Fig. 1.4, right column), while the background EMG activity pattern was similar for all participants.

In contrast, for the ipsilateral BF (iBF), we observed large inter-individual differences both in the background EMG patterns and in the reflexes (Fig. 1.4, left column). Several participants showed significant suppressive reflexes (P1-P3, P5), but for others (P9 and P13) the reflex activity was enhanced in most phases of the gait cycle. These variations did not follow the variations in cTA. In other words a tendency towards facilitation in iBF was not necessarily coupled to a similar tendency in TA (Fig. 1.4). To shed light on the basis of these variations the reflexes were expressed in terms of a maximum gain (reflex/background) and this number was correlated with some of the available characteristics of the subjects (such as body mass, height, body mass index (BMI), as well as with the calculated stability measures). All correlations were below $r = 0.7$ and none reached significance.

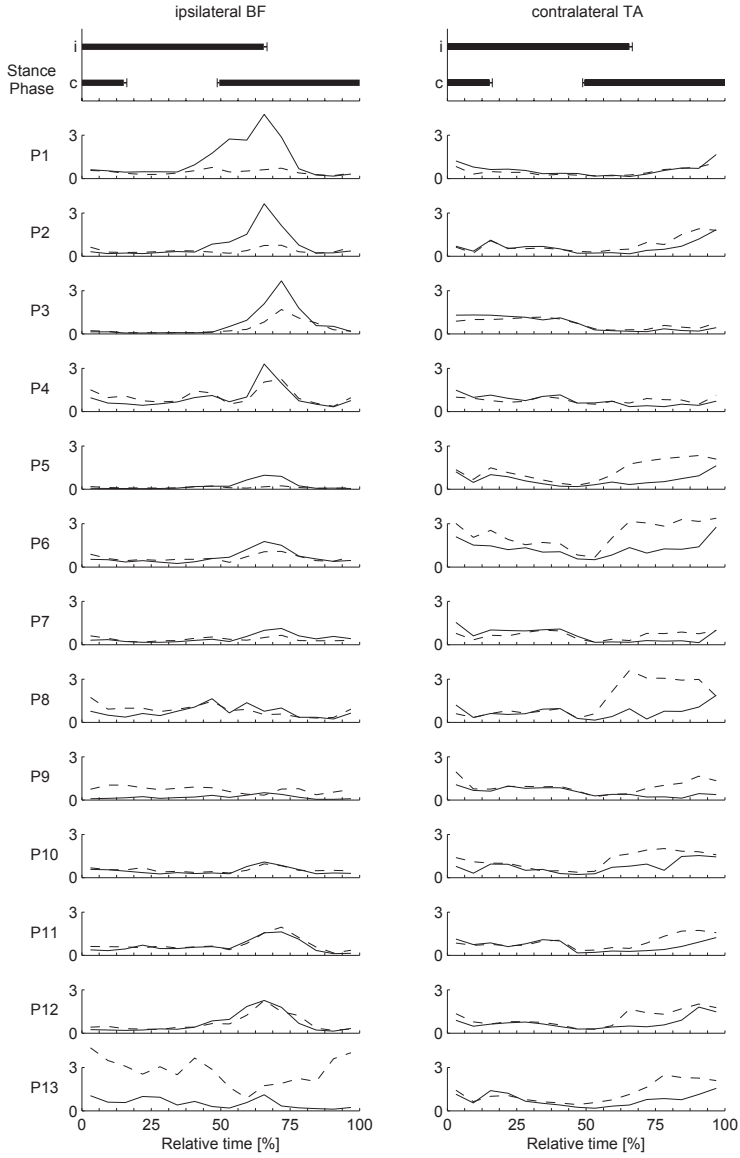


Figure 1.4: ***Background (full line) and reflex (dashed line) EMG values for the ipsilateral biceps femoris (left column) and contralateral tibialis anterior (right column) during backward walking for all individual participants.*** *Top panels indicate the duration of the stance phase for the ipsilateral (i) and contralateral (c) leg; error bars present standard error of the mean.*

1.4 Discussion

In this study, we applied sural nerve stimulation during both forward and backward walking and analyzed the reflex activity in both ipsilateral and contralateral lower limb muscles. The most prominent new observation was the consistent presence of reflex EMG activity in the contralateral TA during the contralateral stance phase during backward walking, while no significant reflex EMG activity was seen in this muscle during the swing phase, although background EMG activity was similar. In addition, we frequently observed significant reflex inhibition in the iBF during the transition from the ipsilateral stance phase to the ipsilateral swing phase (phases 10 & 11; Fig. 1.2). Focusing on the individual participant data of the iBF (Fig. 1.4), large inter-individual differences in both background and reflex EMG patterns can be observed.

In line with earlier observations that backward walking is more variable than forward walking (Choi and Bastian, 2007; Grasso et al., 1998; Hackney and Earhart, 2009; Katsavelis et al., 2010; Kurz et al., 2012; Winter et al., 1989), we observed that BW is less locally stable than FW, i.e. short term maximum Lyaponov exponents were higher for BW. Moreover, step width and the safety margin between the extrapolated center of mass and the base of support was larger for BW. The increased step width and safety margin in BW compared to FW, suggests that participants increased their step width during unstable locomotion to increase the safety margin between the extrapolated center of mass and their base of support (Curtze et al., 2011). Inter-individual differences in the reflex activity in cTA or iBF (as expressed in reflex gain: reflex activity / background activity) were not correlated with any of the stability measures. Thus, while we observed both reduced stability and increased crossed responses in TA during BW, no correlation between these properties was observed. Therefore, no support was found for the proposal that reduced stability in BW would be the cause of pronounced reflex activity in the muscles.

The observation that the reflex activity in the cTA was mostly independent of the level of background activity, suggests that during BW strong 'premotoneuronal' phase dependent modulation of reflexes occurred in this muscle. The source of this modulation is unknown but it is striking that for crossed reflexes in BW, just as for ipsilateral reflexes in FW, the TA was special among the muscles studied, since it consistently showed a large degree of phase-dependent modulation. It may be speculated that this is related to the large contribution of corticospinal projections in the control of this muscle during gait (Dietz, 1992; Duysens et al., 2004). In addition, it is known that stimulation of the cortex induces extra TA activity during

the late swing phase, before heel strike (Pijnappels et al., 1998; Schubert et al., 1997). Transcranial Magnetic Stimulation (TMS) is particularly effective, not only in activating TA but also in controlling sural nerve induced reflexes (Christensen et al., 1999; Nielsen et al., 1997; Pijnappels et al., 1998). Nielsen et al. (1997) applied electrical sural nerve stimulation and TMS in seated participants during tonic voluntary contractions and their results indicated that the TA facilitation was due to increased susceptibility of the corticomotoneuronal cells, rather than by an interaction between the cutaneous and corticospinal volleys at a subcortical site. In gait, similar results were obtained (Pijnappels et al., 1998). In the TA, muscular responses to paired electrical sural nerve and TMS were higher than compared with the linear summation of the two stimuli separately, specifically during the swing phase of the stimulated leg. It needs to be emphasized however that all these data refer to ipsilateral TA and that the actual situation may be more complex for contralateral muscles.

In the ipsilateral BF, the background activity was clearly increased around the stance-swing transition of the ipsilateral leg for several participants (P1-P4; Fig. 1.2). These participants also showed a large reflex inhibition during this period of the gait cycle. For other participants the background activation during the stance-swing transition was less, the accompanying reflex inhibition was less or absent and instead reflex facilitation was observed during the first part of the ipsilateral stance phase (P8, P9, P13; Fig. 1.2).

Data for cutaneous reflexes in the ipsilateral muscles during backward walking is available from a study by Duysens et al. (1996). The group averaged background EMG pattern that we observed (Fig. 1.2), was similar to this earlier study, although we generally observed higher values over the complete gait cycle. The reflex activity pattern was also similar to the earlier study. As a result, our subtracted normalized EMG curve (Fig. 1.2) was similar, but shifted downward, displaying more suppression. The differences between these results of both studies are most likely related to the large inter-individual differences that can be observed in the iBF reflexes. Participants in our study showed varying degrees of iBF reflex inhibition and one participant (P13) showed substantial iBF reflex facilitation, consistent over all phases of the gait cycle.

Substantial inter-individual differences in reflex responses between participants have been reported before (Bagna and Bouyer, 2011; Baken et al., 2006; Duysens et al., 1992). Duysens et al. (1992) observed that most participants displayed a sural nerve TA reflex reversal, but others showed only facilitatory or only suppressive responses over the whole gait cycle. In addition, Bagna and Bouyer (2011) reported that responses to superficial peroneal and tibial nerve stimulation varied across

participants. Baken et al. (2006) grouped six participants and presented the data of the seventh participant separately, as Subject 7 displayed a different modulation in as compared to the remaining population. Also, large individual differences in cutaneous reflexes during gait were observed in cats (Loeb, 1993). Further research focusing on inter-individual differences in background and in reflex EMG is needed, to explore possible causes and relevance of these inter-individual differences.

1.4.1 Other muscles

The responses in the contralateral muscles other than the cTA showed less dramatic phase-dependent modulation during backward walking. For the cGM, reflex activity was similar to the background activity for all phases of the gait cycle. The reflex modulation in the cBF can best be described as a continuous enhancement of the background EMG activity throughout the gait cycle.

As for the iBF, data on the background and reflex EMG patterns of the iTA during BW is available from a study by Duysens et al. (1996). For the iTA, the presently observed reflex modulation pattern was similar, except that we observed less enhancement during the swing phase.

Generally, during FW the background and reflex EMG patterns were similar to those described in earlier studies using non-noxious sural nerve stimulation during walking (Baken et al., 2006; Bastiaanse et al., 2006; Duysens et al., 1992, 2010; Lamont and Zehr, 2007; Van Wezel et al., 1997). Increased reflex activity in the iTA muscle was repeatedly reported at the end of the stance phase and begin of the swing phase, followed by an inhibition at the end of the swing phase (Baken et al., 2006; Bastiaanse et al., 2006; Duysens et al., 1992, 2010; Lamont and Zehr, 2007; Van Wezel et al., 1997). Reflex activity in the iBF commonly resembled the background activity pattern and was enhanced over the complete gait cycle (Baken et al., 2006; Bastiaanse et al., 2006; Duysens et al., 2010; Lamont and Zehr, 2007; Van Wezel et al., 1997). Differences between our results and earlier studies could be caused by small differences in methodology. For example, several studies determined background and reflex EMG activity by averaging over time window of individually determined length (Baken et al., 2006; Bastiaanse et al., 2006; Duysens et al., 2010), while in the current study we applied a 40 ms time window with an individually determined latency.

Less is known about reflex activity in the contralateral leg following sural nerve stimulation. Although reflexes in the cTA were not significant, the subtracted

EMG patterns of the cTA and cBF are in agreement with earlier studies (Duysens et al., 2010; Van Wezel et al., 1997).

1.4.2 Functional relevance

We hypothesized that during backward walking, reduced stability would cause pronounced reflex activity in the muscles of the contralateral leg. We did observe pronounced reflex activity in the contralateral TA; however, this was not due to reduced stability. Alternatively, the pronounced reflex activity might be related to the phase dependent function of the muscles during BW. In the cTA, reflex activity is most pronounced during its single stance phase. In this period the TA resists the horizontal acceleration of the center of mass (Jansen et al., 2012). To evaluate the effect of reflex activity in the TA during single stance during BW we analyzed the induced accelerations due to middle latency responses using a set of kinematic pilot data (At Hof, unpublished observation). This analysis confirmed that reflex activity in the TA during the single stance phase slows down the CoM.

When the sural nerve is activated during the ipsilateral swing phase, a safe strategy would be to slow down. This can be accomplished by increasing activity in the cTA, which is in agreement with the observed pronounced reflex activity. However, these activations are of short duration and were primarily seen in selected muscles such as TA. Hence it is unlikely that they would have large behavioral consequences. This was confirmed after inspection horizontal ground reaction force traces. There were no measurable alterations due to the sural nerve stimulation. It has to be kept in mind that these responses are of small duration and therefore do not always result in large biomechanical changes. A similar situation was observed in a previous study in which auditory perturbations were applied during gait. These resulted in very minor measurable movement changes, thereby allowing for a smooth progression of gait despite clear EMG startle reactions (Nieuwenhuijzen et al., 2000). The present stimulation levels were chosen to be outside the domain of nociception. It is possible, though, that stronger stimuli would yield more dramatic changes, leading to observable decelerations of the CoM. Also, evaluation of (ankle) joint kinematics could provide further insights, similar to the study on startle reactions (Nieuwenhuijzen et al., 2000), but unfortunately no kinematic data was recorded in the current study.

Taken together, the data confirmed that BW was less stable, but no support was found for the proposal that this was the reason for the observed increased crossed responses in TA during BW. Alternatively, the pronounced reflex activity can be

explained by considering the muscle's function in briefly and subtly decelerating the CoM in response to unexpected perturbations.

1.5 Acknowledgements

It is a pleasure to thank At Hof, for his model simulations on a set of pilot data for this project. We thank Marc Beirinckx for developing the software to control the reflex stimulation during the experiment. This work was supported by KULeuven ('bijzonder onderzoeksfonds') Grants OT/08/034 and IDO/07/012 to JD and FWO (grant G.0.756.10.N.10). FM was supported by an F+ fellowship from KULeuven, a Postdoctoral fellowship of the Research Foundation - Flanders (FWO) and a scientific prize of the Foundation Van Goethem-Brichant, Belgium.

2

Cutaneous reflex modulation and self-induced reflex attenuation in cerebellar patients

Published in Journal of Neurophysiology as:
Hoogkamer W, Van Calenbergh F, Swinnen SP, Duysens J. Cutaneous reflex modulation and self-induced reflex attenuation in cerebellar patients. *J Neurophysiol* [Epub ahead of print], 2014.

Abstract

Modulation of cutaneous reflexes is important in the neural control of walking, yet knowledge about underlying neural pathways is still incomplete. Recent studies have suggested that the cerebellum is involved. Here, we evaluated the possible roles of the cerebellum in cutaneous reflex modulation and in attenuation of self-induced reflexes. First it was checked whether leg muscle activity during walking was similar both in patients with focal cerebellar lesions and in healthy controls. We then recorded cutaneous reflex activity in leg muscles during walking. Additionally, we compared reflexes after standard (computer-triggered) stimuli with reflexes after self-induced stimuli for both groups. Biceps femoris and gastrocnemius medialis muscle activity was increased in the patient group compared to the controls, suggesting a co-activation strategy to reduce instability of gait. Cutaneous reflex modulation was similar between healthy controls and cerebellar patients, but the latter appeared less able to attenuate reflexes to self-induced stimuli. This suggests that the cerebellum is not primarily involved in cutaneous reflex modulation but that it could act in attenuation of self-induced reflex responses. The latter role in locomotion would be consistent with the common view that the cerebellum predicts sensory consequences of movement.

Keywords

Cerebellum; ataxia; phase-dependent modulation; locomotion

2.1 Introduction

'Cutaneous reflexes' are seen as (changes in) muscle activity in reaction to non-noxious stimulation of a cutaneous nerve. During gait such reflexes are partly, but certainly not solely, influenced by the ongoing (background) activity in the same muscle (for review: Zehr and Duysens, 2004). This is most clearly seen in the case of a phase-dependent reflex reversal (Duysens et al., 1990; Yang and Stein, 1990). In the tibialis anterior muscle (TA), stimulation of the sural nerve during the early swing phase results in facilitatory reflex activity, while a similar stimulus during the late swing phase results in a suppression of the background activity, despite similar background levels in these two phases (Duysens et al., 1990; Yang and Stein, 1990). Although, this phase-dependent reflex modulation is studied extensively (for review: Zehr and Duysens, 2004), precise knowledge about the underlying neural pathways is still incomplete (Bagna and Bouyer, 2011; Behrendt et al., 2013; Ruff et al., 2014)

In this paper, we aim to evaluate the role of the cerebellum in the modulation of cutaneous reflexes. The cerebellum has an important role in the control of (adaptability of) gait in humans (for review: Ilg and Timmann, 2013; Morton and Bastian, 2007) and observations in rats suggest a role in phase-dependent modulation of cutaneous reflexes as well (Bronsing et al., 2005; Pijpers et al., 2008). Selective impairment of the C1-module in the cerebellum of rats severely affected the modulation of cutaneous reflexes during walking. Whether humans also rely on the cerebellum for this modulation is unknown. Therefore, we analyzed and compared cutaneous reflex activity both in cerebellar patients with stable focal lesions after cerebellar tumor resection and in healthy controls. We hypothesized that phase-dependent reflex modulation patterns would be less pronounced in patients.

Additionally, the cerebellum is thought to be involved in the prediction of sensory consequences of actions (for review: Bastian, 2011; Wolpert et al., 1998). A well-known example is the proposition that the cerebellum is involved in the central cancellation of tickle sensation (Blakemore et al., 1998, 2001). Such predictions are important in the control of movement since afferent feedback has a delay and disruption of cerebellar activity results in movement errors (Miall et al., 2007). Furthermore, this predictive control is also important in active proprioception (Bhanpuri et al., 2013). For locomotion, it has also been suggested that sensory input resulting from one's own movements can be suppressed. In particular, it was found that sensory stimuli from the foot are not easily perceived in the period just after landing, when one may expect an abundance of afferent input from the

foot (Duysens et al., 1995). Reflexes during gait are also suppressed when their occurrence can be predicted because they are elicited voluntarily (Baken et al., 2006). It was hypothesized that the cerebellum would be important for this suppression. To test these hypotheses, we directly compared cutaneous reflexes of cerebellar patients with those of healthy controls in reaction to both externally-triggered and self-induced stimuli.

2.2 Materials and Methods

2.2.1 Participants

Eleven patients with stable focal lesions after cerebellar tumor resection (CBL; age: 24.0 ± 7.1 yrs; 3 males; Table 2.1) and ten healthy participants (age: 23.9 ± 3.7; 5 males) participated in this study. All CBL suffered from cerebellar tumors [medulloblastoma ($n = 4$), pilocytic astrocytoma ($n = 5$), Lhermitte Duclos disease ($n = 1$) or hemangioblastoma ($n = 1$)]. Seven CBL received adjuvant radiotherapy and three of them received adjuvant chemotherapy (treatment details in Table 2.2). In most CBL, no extra-cerebellar damage was seen, neither on MR imaging, nor on clinical examination. In a few CBL (Table 2.2) there were mild signs of extra-cerebellar damage, as assessed on MRI images, but in no case was there a clinical repercussion: three CBL had mild residually enlarged supratentorial ventricular system and a ventriculoperitoneal shunt catheter passing through the right frontal lobe, and three had small sized, asymptomatic cavernous angiomas (Table 2.2). No deficits in muscle force or sensation were observed in any of the CBL during the neurological screening. The reflexes were normal and there were no long tract signs. In the three CBL who had received chemotherapy, there were no signs or symptoms of polyneuropathy (and no data suggestive of polyneuropathy in the medical files at the time chemotherapy was given). CBL were in a stable condition (> 2 years post-op; range $8.7 - 30.2$ yrs) and were able to walk independently. Severity of ataxia was rated using the International Cooperative Ataxia Rating Scale (ICARS) (Trouillas et al., 1997) and scores ranged from 0 to 19 (6.6 ± 5.6; Table 2.1). All participants gave written informed consent. The experiments were conducted in accordance with the Declaration of Helsinki and were approved by the local ethics committee.

Table 2.1: ***Patients were mildly ataxic and in a stable condition (> 2 years post-op)***

#	Age (years)	Time Post-op (years)	Sex	Diagnosis	Interposed Nuclei Lesioned	Adjuvant Therapies Radiation	Adjuvant Therapies Chemo	Lesion Volume (cm^3)	ICARS Total /100	ICARS P&G /34	ICARS Kin Fun /52
1	22.3	17.7	m	Medulloblastoma	Both	Y		22.0	13	6	2
2	18.5	10.0	f	Medulloblastoma	Left	Y	Y	22.6	2	1	1
3	18.6	13.7	m	Medulloblastoma	Right	Y	Y	6.3	19	5	10
4	18.4	15.5	f	Medulloblastoma	Both	Y	Y	5.4	5	1	3
5	31.4	19.7	f	Astrocytoma grade III	Right	Y		8.6	11	5	4
6	21.6	19.5	f	Astrocytoma grade II	Right	Y		7.1	0	0	0
7	26.9	24.9	f	Pilocytic Astrocytoma		Y		58.4	5	1	1
8	19.6	11.8	m	Pilocytic Astrocytoma	Left			1.7	6	3	2
9	20.2	8.7	f	Pilocytic Astrocytoma				8.2	3	0	0
10	28.8	13.9	f	Lhermitte Duclos Disease				58.0	3	1	1
11	39.9	30.2	f	Hemangioblastoma	no MRI			no MRI	6	4	2

Empty fields indicate that no lesions were present or that no adjuvant therapy was received. For patient 11 no MRI data was acquired. f = female, m = male; Y = yes; P&G = Posture & Gait sub-score; Kin Fin = Kinetic Functions sub-score.

Table 2.2: ***Treatment details***

#	Diagnosis	Time Post RT (years)	Target Areas Dose RT (Gray)	Hypo-pituitarism	Time Post CT (years)	Total Duration CT (months)	Scheme CT	VP shunt	Extra-cerebellar sequela
1	Medulloblastoma	17.6	35.2 CSP + 10 SP + 20 FP	Y				Y	Y*
2	Medulloblastoma	9.9	35.2 CSP + 20 FP		8.7	12	HIT-2000	Y	Y**
3	Medulloblastoma	13.1	35.2 CSP + 10 SP + 20 FP	Y	13.0	8	HIT-91	Y	Y**
4	Medulloblastoma	15.2	35.2 CSP + 10 SP + 20 FP	Y	15.2	3	HIT-91		Y***
5	Astrocytoma grade III	18.1	60 FP	Y					
6	Astrocytoma grade II	19.4	50.4 FP						
7	Pilocytic Astrocytoma	24.7	60 FP						Y**
8	Pilocytic Astrocytoma								
9	Pilocytic Astrocytoma							Y	
10	Lhermitte Duclos Disease							Y	
11	Hemangioblastoma							Y	

f = female; m = male; Y = yes; RT = radiotherapy; CT = chemotherapy; CSP = craniospinal; SP = spinal; FP = fossa posterior; CT = chemotherapy; VP = ventriculo-peritoneal; HIT-2000 = cisplatinum, vincristine, CCNU; HIT-91 = ifosfamide, etoposide (VP16), metotrexate, ara-C, cisplatinum.
* Thalamic cavernous angioma, asymptomatic;
** Hydrocephalus;
*** Cavernous angioma parietal white matter, asymptomatic; cavernous angioma intramedullary spinal cord, level D12, 1.8x2.6 mm, asymptomatic.

2.2.2 Experimental set-up and protocol

Procedures and set-up were similar to earlier experiments (e.g. Hoogkamer et al., 2012). Participants walked for ≈10 minutes at 1.11 m/s (4.0 km/hr) on an instrumented dual-belt treadmill (Forcelink, Culemborg, The Netherlands). An electrical stimulus was repeatedly applied at the sural nerve near the ankle of the right leg. The stimulus consisted of a train of 5 rectangular pulses of 1 ms duration with a frequency of 200 Hz (Grass S48 stimulator, in series with an SIU5 isolator and a CCU1 constant-current unit, Grass Instruments).

The stimulation electrode was attached near the lateral malleolus, where the sural nerve is closest to the skin surface (approximately halfway between the lateral malleolus and the Achilles' tendon). We determined the exact position of the electrode according to the optimal irradiation of the stimulus, corresponding to the innervation area of the sural nerve. The stimulus electrode was attached to the skin with tape and stabilized by a strap around the ankle, to keep conditions stable throughout the experiment. We set the stimulus intensity to twice the perception threshold (Duysens et al., 1996). We recorded bipolar EMG in the biceps femoris (BF), tibialis anterior (TA) and gastrocnemius medialis (GM) of both legs by using surface electrodes to sample at 1,000 Hz (ZeroWire, Aurion, Italy). Additionally, ground reaction forces were recorded at 1,000 Hz.

Participants were tested in two different conditions. In the 'externally-triggered' condition the stimuli were automatically triggered by the software. In the 'self-induced' condition the stimuli were manually triggered by the participants, similar to the study by Baken et al. (2006). The order in which participants performed the 'externally-triggered' and 'self-induced' condition was random. In both conditions, custom-written MATLAB software was used to enable a reproducible stimulation at 16 equidistantly distributed phases in the gait cycle. Stimulation was timed relative to the instant of heel strike of the right (stimulated) leg, determined by a vertical force threshold of 10% of body weight. In the 'externally-triggered' condition the software directly triggered the electrical stimuli; in the 'self-induced' condition auditory beeps were generated. We instructed participants to push a handheld button in reaction to the beep. This action triggered a stimulus. We asked the participants to aim for a constant interval between the beep and the button response, and we emphasized it was not necessary to respond as fast as possible (as in a reaction time test) (Baken et al., 2006). This was important since the aim was to have the participants perform a voluntary movement, unaffected by start-react effects. The algorithm presented stimuli/beeps in each phase in random order, such that there was at least one complete stride without a stimulus between

consecutive stimuli. To reach the target of 10 stimuli in each of the 16 phases, 15 beeps were presented per phase (to account for variability in reaction time).

2.2.3 Data analyses

Data analysis procedures were similar to Hoogkamer et al. (2012). Raw force data was filtered with a fourth-order recursive, zero phase-shift, Butterworth low-pass filter with a cutoff frequency of 10 Hz. Instants of heel strike and toe-off were determined, based on anterior-posterior and medio-lateral maxima in the center of pressure trajectory (Roerdink et al., 2008). Stride time and stance percentages were calculated based on the instants of heel strike and toe-off. Variability of these parameters was assessed using the coefficient of variation (CV); the ratio between the standard deviation and the mean values. We defined gait cycles from right heel strike (0%) to the next right heel strike (100%). We excluded strides when a foot incidentally was placed on two belts or when two feet were on the same belt.

The EMG signals were amplified and high-pass filtered (cutoff frequency 3 Hz), full-wave rectified and low-pass filtered (cutoff frequency 300 Hz). To evaluate muscle activity and TA-GM co-activation over the gait cycle, EMG traces were time-normalized into 100 samples per gait cycle. For these analyses only the strides without stimuli and reflex responses were included. For each muscle the average muscle activity over these strides was calculated and then normalized to its maximum value during the gait cycle. Finally, the normalized traces of both legs were averaged. TA-GM co-activation was calculated sample by sample based on the normalized muscle activity in these muscles using:

$$\text{TA-GM co-activation} = \frac{a_H + a_L}{2} \times \frac{a_L}{a_H}$$

where a_H represents the activity of the muscle that has the highest activity during the considered sample (i.e. either TA or GM) and a_L represents the activity of the other muscle at the same time sample (Mari et al., 2014).

We quantified the reflex responses by calculating the mean of the EMG data over the reflex time window. Reflex time windows were manually set around the middle latency reflexes (or 'P2 reflexes'), starting $70 - 80$ ms after the stimulation (Baken et al., 2005; Duysens et al., 1993; Haridas et al., 2005; Yang and Stein, 1990). The time windows were estimated based on visual inspection of the subtracted EMG traces (see below) (Duysens et al., 1996). A single time window, relative to the stimulus, per muscle was used for all conditions. For muscles showing little or no response, we set a time window based on the response in other muscles (e.g. Duysens et al., 1996, 2010; Tax et al., 1995; Van Wezel et al., 1997). Time window

mean values were designated to the appropriate phases based on the onset of the response (start of the time window).

For each of the 16 phases of the gait cycle we performed the following calculations to get the reflex and background EMG data of that phase: we averaged the values of 10 stimulated strides (Fig. 2.1). For each stimulus, we estimated the background EMG activity by calculating the mean EMG over the same relative time window of the preceding stride (without stimulation). Next, we calculated the average value of the 10 unstimulated strides. Finally, we averaged the background values from the two conditions.

Then, background and reflex EMG data was normalized to the maximum background EMG activity (phase-average) for each participant and muscle. In a last step, we then calculated the 'net' reflex amplitude by subtracting the background EMG activity from the reflex EMG activity (Fig. 2.1). The range of this subtracted reflex curve was used as an index of reflex modulation.

To assess habituation in the reflex responses, we analyzed the mean absolute reflex responses to self-induced stimuli over all phases of the gait cycle, per stimulus number. For each participant, these responses were then normalized with respects to their average. Eventually, group means were calculated and ordered according appearance during the trial.

2.2.4 MRI data acquisition and processing

Image acquisition was performed using a Philips 3T Achieva MRI scanner (Philips, Best, The Netherlands) with a 32-channel matrix head coil. For all but one CBL, a 3D MPRAGE high resolution T1-weighted image (repetition time = 970 ms, echo time = 4.60 ms, flip angle = 8°, 230 1-mm slices, in-plane resolution = 0.97×0.98, 384×384 matrix) was acquired.

Lesions on the MPRAGE images were manually traced using MRIcroN software (http://www.mccauslandcenter.sc.edu/mricro/mricron/index.html). Lesion traces were spatially normalized to the atlas of the cerebellum (Diedrichsen et al., 2009) using the SUIT toolbox (http://www.icn.ucl.ac.uk/motorcontrol/imaging/suit.htm Diedrichsen, 2006; Diedrichsen et al., 2011) in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). When spatial normalization with the SUIT toolbox was inaccurate (some cases with large lesions at the outer border of the cerebellum), lesions were spatially normalized based on the whole brain image with manual corrections in atlas space when needed. Total lesion size was assessed (Table 2.1) and lesions in the interposed (Table 2.1) and other deep cerebellar nuclei (Table 2.3)

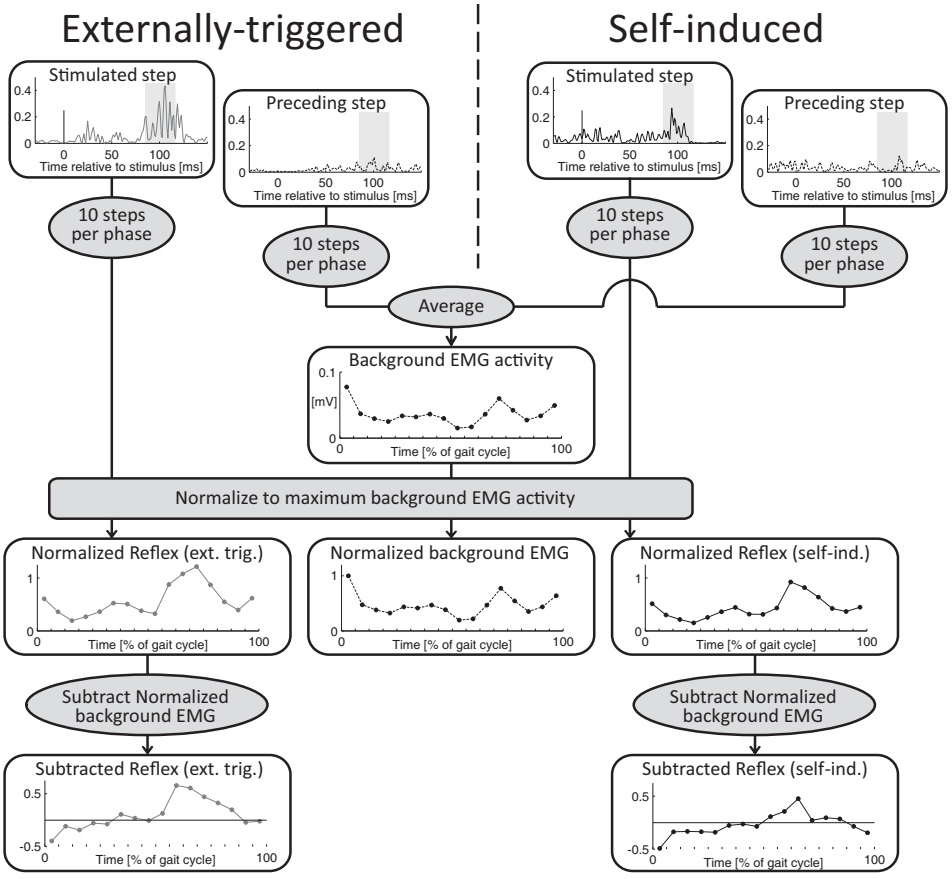


Figure 2.1: ***Schematic overview of the EMG data analysis procedures.*** *Top panels display rectified, low pass filtered EMG activity. In each panel the time axis is based on the timing of the stimulus (stimulated step) or the same relative timing in the preceding gait cycle (unstimulated steps). Full lines represent data for stimulated steps; dashed lines represent data for unstimulated steps. Grey shading on EMG traces represents the time window, over which average EMG activity values were calculated. For each phase of the gait cycle we averaged the values of 10 steps. Background EMG traces were then calculated as the average of the background traces of the 'Externally-triggered' condition (left hand side) and the 'Self-induced' condition (right hand side). Then, background and reflex EMG data was normalized to the maximum background EMG activity; displayed in the three panels with Normalized activity. Grey lines represent traces for the 'Externally-triggered' condition (left hand side); black lines represent traces for the 'Self-induced' condition (right hand side). In a last step, we then calculated the 'net' reflex amplitude by subtracting the background EMG activity from the reflex EMG activity.*

Table 2.3: ***Overview of lesioned lobules and nuclei***

	Vermis								Paravermis									
#	VI	CI	CII	VIIb	VIIIa	VIIIb	IX	X	I-IV	V	VI	CI	CII	VIIb	VIIIa	VIIIb	IX	X
1					Y	Y	Y	Y	L	B			B	B	B	B	B	
2	Y	Y	Y	Y	Y	Y	Y		B	B	L	L	B	B	B	B	B	
3					Y	Y	Y	Y					R	R	R	R	B	
4	Y		Y	Y	Y	Y	Y	Y					B	B			B	
5					Y	Y	Y	Y						R	R	R	R	R
6	Y		Y	Y	Y	Y	Y				R	R	R	B	R	R	R	
7											L	L	L	L	L			
8			Y	Y	Y	Y	Y						L	L	L	L	L	
9																		
10									L	L	L	L	L	L	L	L		
11	no MRI								no MRI									

	Hemispheres									Nuclei		
#	I-IV	V	VI	CI	CII	VIIb	VIIIa	VIIIb	X	F	I	D
1					L	L				B	B	B
2		L	L							B	L	B
3											R	B
4										R	B	B
5										R	R	
6											R	B
7			L	L	L	L	L					
8											L	
9				R	R	R	R	R				
10	L	L	L	L	L	L	L	L				
11	no MRI									no MRI		

For patient 11 no MRI data was acquired; Y = yes; L = left; R = right; B = both; F = fastigial nuclei; I = interposed nuclei; D = dentate nuclei.

were listed (see Fig. 2.2 for superposition image of all lesions). A probability threshold of 20% was used to determine whether the normalized lesions overlapped with the specific nuclei (Diedrichsen et al., 2011) and lobules.

2.2.5 Statistical analyses

Results in the text are presented as mean values $\pm SD$. To compare muscle activity and co-activation during the normal (unstimulated) strides between groups, we calculated the mean values of these parameters during four periods of the gait cycle: initial double stance, single stance, terminal double stance and swing. A group $\times$ period ANOVA was performed for each muscle and for the TA-GM co-activation. To evaluate potential differences in phase-dependent modulation of subtracted reflex responses between groups, we used Mann-Whitney U tests (Baken et al., 2006; Duysens et al., 2010; Van Wezel et al., 1997). To evaluate the cerebellar role in attenuation of self-induced reflex responses, we compared subtracted reflex EMG in the TA muscle after externally-triggered and self-induced stimuli within groups (Wilcoxon Matched Pairs Test). In addition to these tests

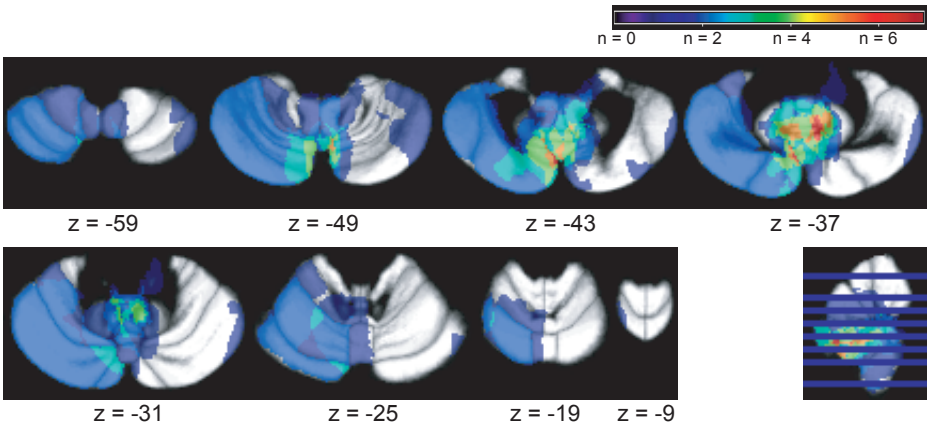


Figure 2.2: ***Superposition of the regions of cerebellar lesions of all patients.*** *Maximum overlap (7 patients) was within the left paravermal lobule VIIb.*

(uncorrected for multiple comparisons), we performed a group × condition ANOVA on average values over the period where suppression was relevant ($43.75 - 93.75\%$ of the gait cycle). To assess correlations between muscle activity, reflex attenuation, temporal gait parameters and ICARS (sub) scores, Spearman's rank correlation coefficients were calculated. Additionally, we used Wilcoxon tests to assess whether muscle activity and reflex attenuation was different between patient subgroups (subdivisions based on lesion characteristics; Table 2.1). A traditional level of significance was used for all statistical tests ($\alpha = 0.05$).

2.3 Results

2.3.1 Muscle activity during gait

Stride time (CBL: 1.05 ± 0.03 s vs. healthy controls: 1.07 ± 0.07 s, $p = 0.55$) and relative stance time ($64.6 \pm 0.7\%$ vs. 64.6 ± 0.5, $p = 0.60$) were similar between groups, but variability of those parameters was higher in the CBL group than in the control group (stride time variability: $2.6 \pm 0.8\%$ vs. $1.6 \pm 0.2\%$, $p < 0.001$; relative stance time variability: 2.3 ± 0.7 vs. $1.4 \pm 0.2\%$; $p < 0.001$). CBL walked with increased activity in the BF muscles, specifically during the single stance period (Fig. 2.3; main effect for group: $p = 0.002$; group × period interaction: $p = 0.027$; post hoc comparisons: initial double stance $p = 0.67$, single stance

$p = 0.002$, terminal double stance $p = 0.08$, swing $p = 1.0$). For the GM muscle a significant main effect for group indicated that the CBL grouped walked with a higher activation than the healthy control group ($p = 0.042$). The group $\times$ period interaction was not significant ($p = 0.07$). For the TA muscle the main effect for group and the group $\times$ period interaction were not significant ($p = 0.43$ and $p = 0.61$, respectively). The main effect for period was significant for all muscles ($p < 0.001$).

Co-activation between the TA and GM muscles appeared higher in the CBL group, specifically during the double stance periods and during the swing, but no significant main effect for group ($p = 0.06$) or group $\times$ period interaction ($p = 0.32$) was observed (Fig. 2.3). Overall co-activation was small in both groups (< 0.2). To assess whether the increased co-activation or the increased BF activity in the CBL group was related to ataxia severity or gait variability, we correlated these muscle activation measures with ICARS (sub) scores and variability of either stride time or relative stance time. None of those parameters were significantly correlated ($rho < 0.5; p > 0.10$, for all). In addition, we compared TA-GM co-activation and BF activity between subgroups based on lesion location (affected nuclei) or radiation therapy application. No differences were observed (all $p > 0.10$).

2.3.2 Phase-dependent reflex modulation

Based on the difference in background EMG one would expect to see larger BF responses in the cerebellar group. This appears to occur for the contralateral BF (cBF; Fig. 2.4), but not for the ipsilateral side where there was no increase in BF responses during most of the stance phase despite the consistent increase in background activity. However, specifically for the BF inter-individual variability was large (see also individual indexes of reflex modulation; Fig. 2.4, right panels). As such no significant group differences were observed in subtracted reflex traces of the BF muscles. In the GM one would have expected some larger responses in the second half of the step cycle (increased background) in the patients, but this was not observed, at least not in the ipsilateral GM (iGM; Fig. 2.4). On the contralateral side there was significantly less suppression during the late single stance in the patient group while background did not differ ($87.5 - 93.75\%$ of the gait cycle of the stimulated limb). In TA the subtracted reflex EMG traces were similar between groups (Fig. 2.4). In both groups the iTA traces showed a reflex reversal with clear facilitatory reflex activity during the early swing phase and suppressive activity around heel strike. For all muscles the indexes of reflex modulation were similar between groups (Fig. 2.4, right panels).

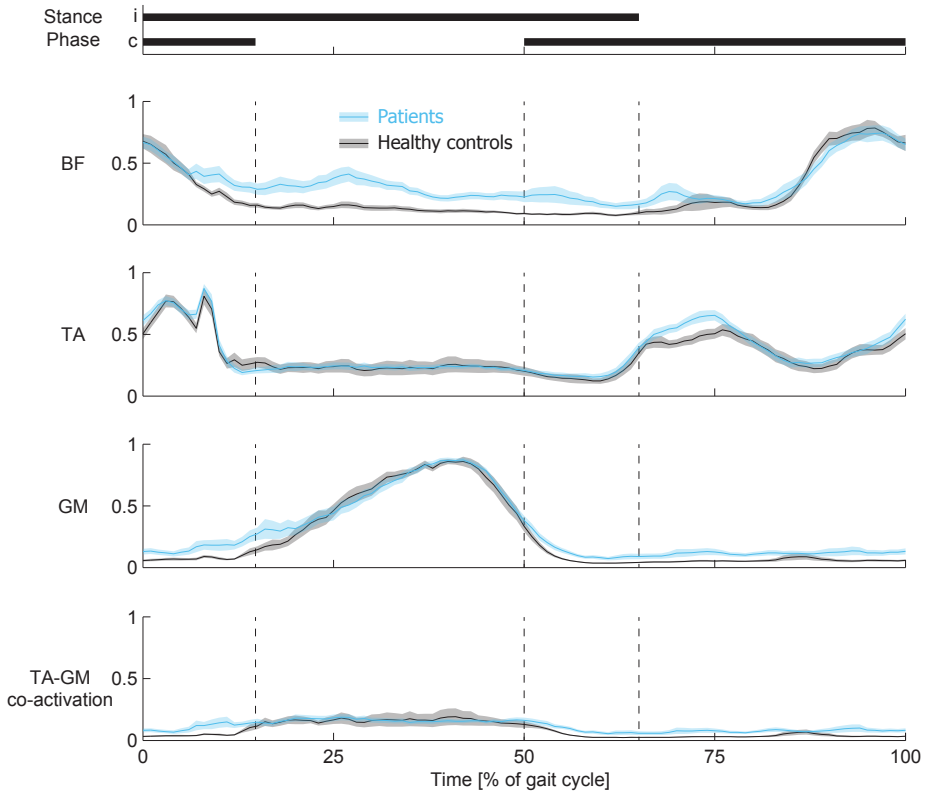


Figure 2.3: ***Comparisons of muscle activity and co-activation traces between cerebellar patients and healthy controls.*** *Muscle EMG activity for cerebellar patients (CBL; blue line) and healthy controls (black line). Shaded areas show SE. Duration of the stance period for the ipsilateral (i) and contralateral (c) legs are displayed in the top frames. BF, biceps femoris; TA, tibialis anterior; GM, medial gastrocnemius. Bottom panel: Co-activation between TA and GM for cerebellar patients (blue line) and healthy controls (black line). Co-activation was calculated sample by sample based on the normalized muscle activity (mean of both legs) using: TA-GM co-activation* $= \frac{a_H + a_L}{2} \times \frac{a_L}{a_H}$*, where* a_H *represents the activity of the muscle that has the highest activity during that phase (i.e. either TA or GM) and* a_L *represents the activity of the other muscle during that phase (Mari et al., 2014).*

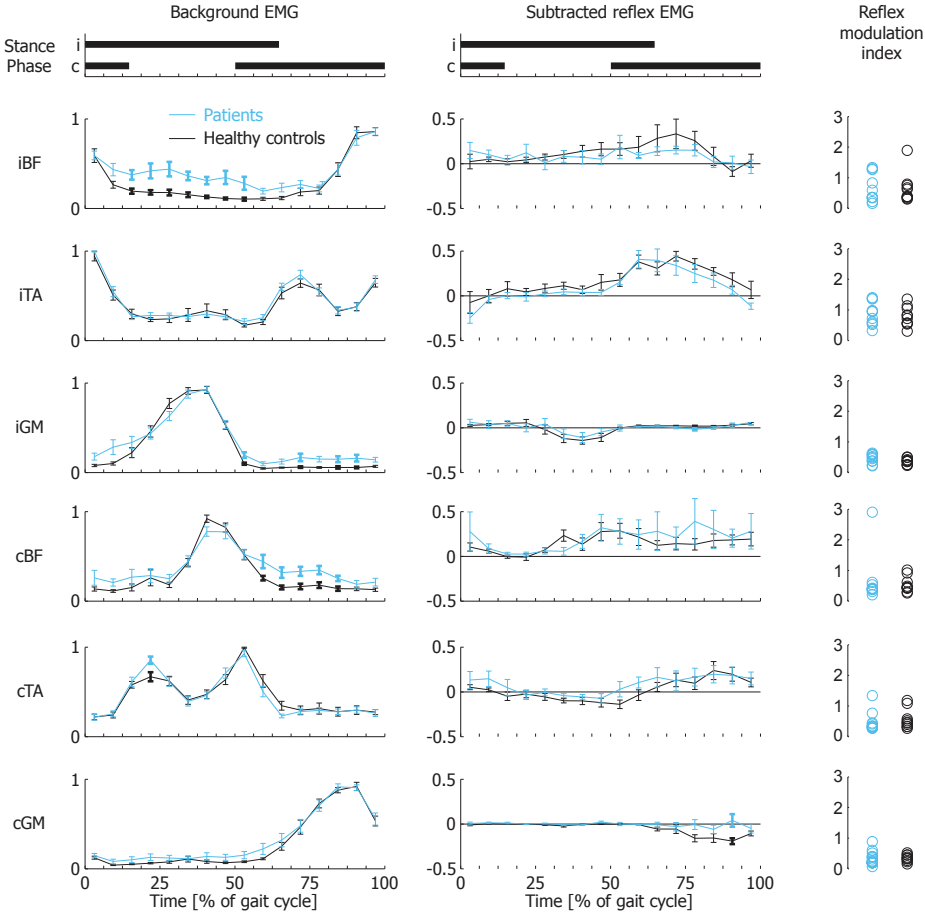


Figure 2.4: ***Subtracted reflex traces and modulation indexes are similar between cerebellar patients and healthy controls.*** *Normalized background EMG activity (left) and normalized subtracted reflex EMG activity (middle) for cerebellar patients (CBL; blue line) and healthy controls (black line). Error bars show SE. Bold data points and error bars indicate phases when CBL differ significantly from healthy controls in a given phase (uncorrected for multiple comparisons). Durations of the stance period for the ipsilateral (i) and contralateral (c) legs are displayed in the top frames. Reflex modulation index (right) in different muscles for CBL (blue circles) and healthy controls (black circles). BF, biceps femoris; TA, tibialis anterior; GM, medial gastrocnemius. Note that subtracted reflex traces and modulation indexes are similar between CBL and controls, irrespective of differences in background EMG.*

2.3.3 Attenuation of self-induced reflex responses

To evaluate attenuation of self-induced reflex responses (Fig. 2.5), we compared subtracted reflex EMG in the iTA muscle after externally-triggered and self-induced stimuli, since the mean reduction in reflex responses is known to be the strongest in the iTA Baken et al. (2006). Background EMG activity was similar between groups for this muscle (as mentioned above; Fig. 2.3). However the suppression of reflex responses showed a tendency to differ between the two groups (Fig. 2.5). In the control group, reduced subtracted reflex responses were seen in the stance swing transition and during the swing phase. Specifically, this reflex activity was attenuated for several phases when self-induced ($56.25 - 62.5$, $68.75 - 81.25$ and $87.5 - 93.75\%$ of the gait cycle). This attenuation was less pronounced in the CBL group where reflex activity to externally-triggered and self-induced stimuli only differed during the early stance phase of the contralateral leg ($50 - 56.25\%$ of the gait cycle; Fig. 2.5). To evaluate whether this attenuation was different between groups we calculated the mean subtracted reflex activity over the period where suppression could occur (namely $43.75 - 93.75\%$ of the gait cycle) for both conditions in both groups (Fig. 2.5) and performed a repeated measures ANOVA. The majority of the CBL and all healthy controls showed attenuation of self-induced reflex responses (Fig. 2.5, bottom panel) and a significant main effect for condition was observed ($p < 0.001$), indicating that in general reflexes to self-induced stimuli were smaller than reflexes to externally-triggered stimuli. However, the main effect for group was not significant ($p = 0.69$) and also the group $\times$ condition interaction did not reach significance ($p = 0.14$). Hence, attenuation of self-induced reflex responses appeared only different between groups when considering individual phases but not when considered over the whole period. The amount of suppression varied between CBL, but there was no correlation with ICARS (sub) scores, lesion location (affected nuclei) or radiation therapy application for this relatively small group (all $p > 0.10$).

2.3.4 Habituation

To assess whether differences in attenuation of self-induced reflexes were related to different habituation characteristics for both groups, we plotted the mean responses with respect to order of presentation (Fig. 2.6). Habituation was similar between groups (Fig. 2.6) and habituation of responses was very modest, conform to previous studies (Bastiaanse et al., 2006, see also figure 6 in Tax et al. 1995).

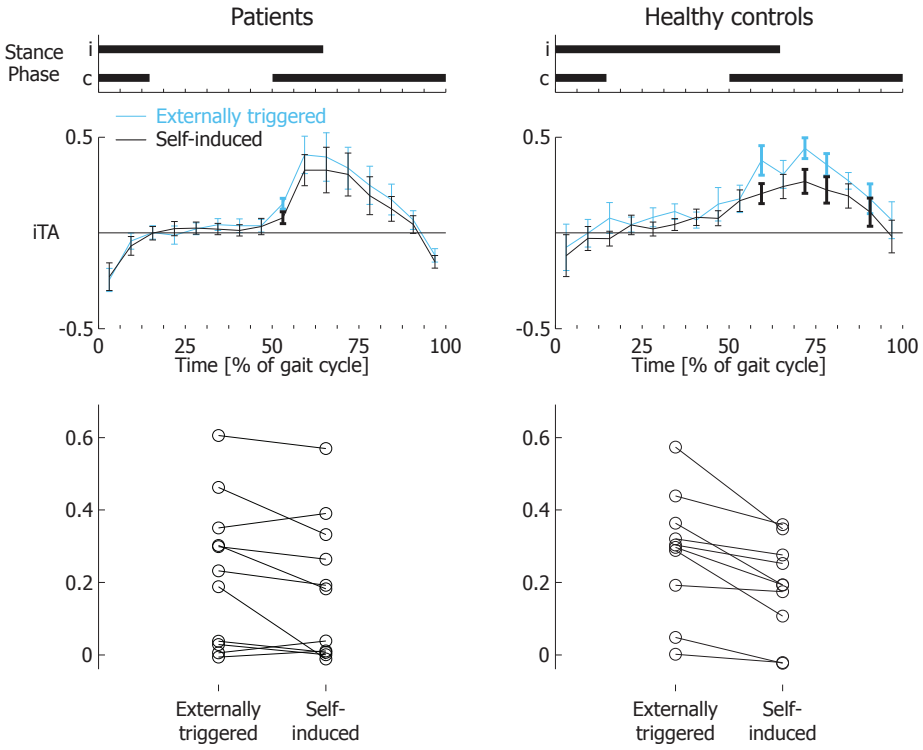


Figure 2.5: ***Attenuation of self-induced reflex responses in cerebellar patients and healthy controls.*** *Normalized subtracted reflex EMG activity for cerebellar patients (left) and healthy control (right). Durations of the stance periods for the ipsilateral (i) and contralateral (c) legs are displayed in the top frames. Middle frames: Self-induced reflex are shown in black, externally-triggered reflexes in blue. Error bars show SE. Bold data points and error bars indicate phases when self-induced reflex activity is significantly attenuated. iTA, ipsilateral tibialis anterior. Bottom frames: Individual data for externally-triggered and self-induced reflexes.*

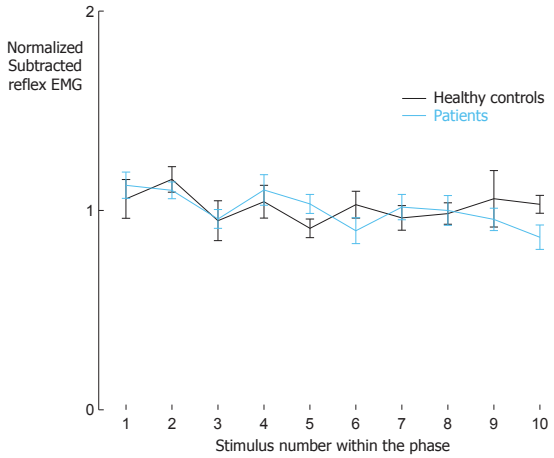


Figure 2.6: ***Limited habituation across trials.*** *Total mean reflex responses due to self-induced stimuli as a function of the order of appearance during the trial for cerebellar patients (blue line) and healthy controls (black line). We analyzed the mean absolute reflex responses to self-induced stimuli over all phases of the gait cycle, per stimulus number. For each participant, these responses were then normalized with respect to their average. Eventually, group means were calculated and plotted as a function of the order of appearance during the trial. Error bars show SE.*

2.4 Discussion

In this study we aimed to address the role of the cerebellum in modulation or attenuation of cutaneous reflexes during gait. We observed that patients with focal lesions in the cerebellum walk with an overall higher BF and GM muscle activity than healthy controls. Our results from the reflex activity analyses show relatively minor differences (with respect to the differences in background, some expected increases in reflexes were not seen in some muscles such as BF and GM). Hence the data do not suggest that the cerebellum has a major role in phase-dependent modulation of cutaneous reflexes in walking humans. On the other hand, the suppression of reflexes, which was consistently seen in controls, was much less apparent in the patients (less phases with significant suppression). Hence these data do suggest that the cerebellum might play a role in attenuating cutaneous reflexes after self-induced stimuli.

2.4.1 Increased GM and BF activity

Although the evaluation of the muscle activity during the steps without stimulation was not a main goal in this study, the first major finding was that the CBL group walked with an overall higher GM and BF muscle activity than healthy controls. The increased GM muscle activity was accompanied with an overall increased TM-GM co-activation. BF muscle activity was specifically higher during the single stance period. Muscle activity has been observed to be increased in more severely affected cerebellar ataxia patients with diffuse cerebellar damage (Mari et al., 2014; Mitoma et al., 2000). In those patients, the increased activity in the GM and BF muscles was accompanied by an increased activity in their antagonists, the TA and vastus lateralis muscles, respectively. Such antagonist co-activation could increase leg stiffness, which can be a compensation strategy when postural threat is elevated. It is often observed in walking elderly (Franz and Kram, 2013; Hortobágyi et al., 2009; Peterson and Martin, 2010). In our CBL group TA-GM co-activation was higher overall, similar to observations by Mari et al. (2014) for more severely affected cerebellar ataxia patients with diffuse cerebellar damage. As our main purpose was to evaluate cutaneous reflex activity, we did not measure EMG activity in the vastus lateralis. Accordingly, we could not directly assess co-activation between the BF and the vastus lateralis in our patient group.

2.4.2 Phase-dependent reflex modulation

Increased co-activation could theoretically lead to increased reflex activity, due to increased excitability of the motoneuron pool. Indeed minor differences were observed for the GM muscles, but for the BF and TA muscles no significant differences were observed in the subtracted reflex traces of both legs. Overall, the reflex modulation patterns of both groups were similar to those observed in earlier studies (Baken et al., 2006; Bastiaanse et al., 2006; Duysens et al., 2010; Hoogkamer et al., 2012; Lamont and Zehr, 2007; Van Wezel et al., 1997). Hence overall the results point towards subtle changes in some muscles but overall there is no strong indication that the cerebellum plays a dominant role in the modulation of reflexes.

At first sight the present results appear to be inconsistent with animal studies. In rats, Pijpers et al. (2008) observed reduced reflex activity in the BF after selective impairment of the C1-module in the cerebellum. However, it should be mentioned that the present group consisted of mildly affected patients with long standing lesions. Lesions in our CBL group were due to tumor resection at young

age (7.1 ± 4.3 yrs) and their immature brains had a high potential for neural reorganization and compensation (Caeyenberghs et al., 2009; Gramsbergen, 2007; Kolb et al., 2001). In contrast, the rats in the study mentioned had acute lesions and had no chance to show plastic changes. Furthermore, it should be noted that in the rat study the differences in reflexes between lesioned rats and control rats became less apparent and non-significant at postoperative day 7. Finally, it could be that the locations of the lesions differed between the rats and the humans. In the rats the hind-limb part of C1-module (paravermal zones of lobules I-IV, V and VIII; medial part of the anterior interposed nucleus) was impaired (Pijpers et al., 2008). In none of the CBL these paravermal zones of lobules I-IV, V and VIII were fully affected (Table 2.3). However in most CBL the interposed nuclei were affected (Table 2.1). Hence, if these areas were important for this function one would have expected to see at least some effect.

A second point is that, in humans, differences between populations are difficult to demonstrate because there are considerable inter-individual differences with respect to the modulation of this type of reflexes (Bagna and Bouyer, 2011; Baken et al., 2006; Duysens et al., 1992; Hoogkamer et al., 2012; Ruff et al., 2014). Due to the nature of our patient sample, one should be cautious in drawing too strong conclusions. Related to the potential for compensation mentioned above, and the limited size of the lesions and the variation in their location, it could be argued that cerebellar damage in our sample was too mild to observe major effects on phase-dependent reflex modulation.

In this respect it would be interesting to evaluate phase-dependent modulation of cutaneous reflex in more severe ataxic patients, such as patients with degenerative cerebellar diseases. However, gait pattern and muscle coordination in these patients might be too much affected, indirectly changing reflex modulation, and making it less feasible to assess the direct effects of the cerebellar damage on reflex modulation. Future research should also focus on other brain structures to reveal the underlying neural pathway for phase-dependent reflex modulation. So far, the most likely neural structures involved are the spinal cord (Central Pattern Generator) and the motor cortex (for review: Duysens et al., 2004). Results from several transcranial magnetic stimulation studies suggest an important role for the motor cortex and the transcortical pathway (Christensen et al., 1999; Nielsen et al., 1997; Pijnappels et al., 1998). Recent observations of phase-dependent reflex modulation during passive viewing of walking (Behrendt et al., 2013) and during visually guided stepping (Ruff et al., 2014) further support the importance of the motor cortex in reflex modulation.

2.4.3 Attenuation of self-induced reflex responses

Our third finding entails the observation that some CBL were less able to attenuate reflex activity after self-induced stimuli than healthy controls. There was a clear reduction in the number of phases where such suppression was observed. Attenuated reflexes after self-induced stimuli during walking were first observed by (Baken et al., 2006). In their main group, self-induced reflexes were attenuated over 50% of the gait cycle, from mid stance to mid swing ($31.25 - 81.25\%$ of the gait cycle). Healthy participants in our study showed attenuated reflexes over 25% of the gait cycle, from late stance to mid swing ($56.25 - 62.5$, $68.75 - 81.25$ and $87.5 - 93.75\%$ of the gait cycle; Fig. 2.5). In the patient group attenuation occurred in a single phase of the gait cycle during double stance ($50 - 56.25\%$ of the gait cycle). However, on a group level (considering the period where suppression could occur) the reflex attenuation was not significantly different between groups. Hence, one has to conclude that the data are suggestive for a contribution of cerebellar structures but that a definite role cannot be assigned based on the present sample of mildly affected cerebellar patients. Note that we specifically refer to these reflexes as 'self-induced' since it was observed in earlier work that auditory cues preceding the stimuli do not result in reflex attenuation, suggesting that the attenuation is not just caused by anticipation of the stimuli in general (Bastiaanse et al., 2006).

Cerebellar involvement in reflex suppression would be in line with findings from other studies, supporting the idea that the cerebellum provides signals to cancel the sensory response to self-induced stimulation. According to this idea, predictions of the sensory consequences of motor commands are used to partly cancel the actual sensory consequences. This would make the system more sensitive to external (unexpected) perturbations as they result in unpredictable sensory inputs. Furthermore, such predictions are an important control feature. Without predictive control a system only depends on feedback information, which comes with delays, making correction of movements in real time impossible (Miall et al., 2007; Wolpert and Flanagan, 2001). Reduced responses after self-induced stimuli or self-initiated actions have been observed in multiple different domains, such as self-induced tickling sensations (Blakemore et al., 1998, 2001), active head movements (McCrea et al., 1999), self-induced muscle stimulation (Gerilovsky et al., 2002), self-induced sounds (Knolle et al., 2013) and self-induced eye blink reflexes (Meincke et al., 2003). In the latter study it was found that the electrically evoked R2 blink reflex is suppressed by using self-stimulation (in particular the later sections of R2 were affected). The current study shows that such self-induced suppression can be consistently evoked in the context of cutaneous reflexes during locomotion. Note that habituation was similar between groups, which suggests that differences

in attenuation of self-induced reflexes were not related to different habituation characteristics.

2.5 Acknowledgements

We thank Karen Jansen and Marc Beirinckx for developing the software to control the reflex stimulation during the experiment. This work was supported by Research Foundation-Flanders (FWO grant G.0756.10). JD has been funded by Research Foundation-Flanders (FWO grant G.0901.11) and by the Interuniversity Attraction Poles Program initiated by the Belgian Science Policy Office (P7/11).

3

Towards new sensitive measures to evaluate gait stability in focal cerebellar lesion patients

Submitted to Gait & Posture:
Hoogkamer W, Bruijn SM, Sunaert S, Swinnen SP, Van Calenbergh F, Duysens J. Towards new sensitive measures to evaluate gait stability in focal cerebellar lesion patients.

Abstract

The evident ataxic characteristics of gait in patients with cerebellar damage suggest that the cerebellum plays an important role in the neural control of gait. Ataxic features, such as increased gait variability and increased step width, are often related to gait stability. However, the link between these measures and gait stability is not straightforward. Therefore, to gain more insights into relations between gait stability, gait variability and gait ataxia, we quantified gait stability using the short-term maximum Lyapunov exponent. This is a more valid measure of gait stability, derived from dynamical systems theory. Eighteen patients with focal cerebellar lesions after tumor resection walked on an instrumented treadmill at 1.0 m/s for three minutes. The patients displayed relatively mild functional deficits ($ICARS = 6.9 \pm 6.4$, range $0 - 20$) and had a lower overground walking speed as compared to healthy controls (1.12 m/s versus 1.31 m/s). During treadmill walking, the short-term maximum Lyapunov exponent was higher in cerebellar patients, indicating reduced gait stability. Furthermore, step width was increased in the patient group while other spatio-temporal gait parameters were similar. Patients with the largest lesions in the vermis displayed the least stable gait pattern. These observations confirm the cerebellum's importance in the supraspinal control of gait in humans, specifically in relation to gait stability.

Highlights

- Gait stability was quantified using the short-term maximum Lyapunov exponent
- Gait stability was reduced in mildly ataxic patients with focal cerebellar lesions
- Vermal lesion size correlated with gait stability (Lyapunov exponent)
- Step width was increased in the patients and was correlated to ataxia severity

Keywords

Ataxia; cerebellum; locomotion; short-term maximum Lyapunov exponent; vermis

3.1 Introduction

The evident ataxic characteristics of gait in patients with cerebellar damage suggest that the cerebellum plays an important role in the neural control of gait (for review: Ilg and Timmann, 2013). Prominent ataxic gait features include increased gait variability (Ilg et al., 2007, 2008, 2013; Palliyath et al., 1998; Schniepp et al., 2012, 2014; Serrao et al., 2012; Stolze et al., 2002; Wuehr et al., 2013) and an increased step width (Ilg et al., 2007, 2008; Palliyath et al., 1998; Serrao et al., 2012; Stolze et al., 2002). Increased gait variability is often used to infer reduced gait stability (for review: Bruijn et al., 2013) and, similarly, step width has been used as a measure of gait stability, for instance in cerebellar lesion patients (Ilg et al., 2008). In this group increased step length variability has been associated with damage in specific areas in the cerebellum, partially different from cerebellar areas related to increased lateral sway and step width (Ilg et al., 2008, 2013). These observations are important since the individual contributions of balance and limb-coordination deficits to ataxic gait are still under debate (Ilg et al., 2007; Morton and Bastian, 2003). Furthermore, increased gait variability, specifically during slow walking, has been linked to fall risk in cerebellar ataxia patients (Schniepp et al., 2014).

However, the link between gait variability and gait stability is not straightforward. From a biomechanical perspective, increased variability itself does not necessarily imply decreased stability (Bruijn et al., 2013). This notion is important since in cerebellar patients increased gait variability could be related to cerebellar deficits in intra-limb coordination (Ilg et al., 2007), rather than to gait stability.

Likewise, step width is also not an ideal measure to quantify gait stability. In order not to fall, the center of mass needs to be controlled such that it stays over the base of support. For dynamic conditions, such as gait, this is best assessed using the extrapolated center of mass concept (Hof et al., 2005). The extrapolated center of mass combines center of mass position and velocity, and it should be within the base of support (Bruijn et al., 2013; Hof et al., 2005). Because the distance between the extrapolated center of mass and the boundary of the base of support (the 'margin of stability'), is not only affected by step width but also by movements of the rest of the body, it is clear that an increased step width does not necessarily imply more stability.

A more valid (Bruijn et al., 2013) measure for gait stability is the short-term maximum Lyapunov exponent (Dingwell and Cusumano, 2000), derived from dynamical systems theory. This measure quantifies the ability to recover from small perturbations. It has a valid theoretical basis and has been shown to have a high predictive validity in both modeling and observational studies (for review:

Bruijn et al., 2013). While this measure has been used to evaluate gait stability in many different populations such as elderly (Kang and Dingwell, 2009; Toebes et al., 2012), amputees (Lamoth et al., 2010; Segal et al., 2010) and patients with knee osteoarthritis (Yakhdani et al., 2010), anterior cruciate ligament deficiency (Stergiou et al., 2004) and peripheral neuropathy (Manor and Li, 2009), so far it has, to the best of our knowledge, not been used to evaluate gait stability in cerebellar ataxia patients.

Here, we aimed to gain more insight into relations between gait stability, gait variability and gait ataxia. Therefore, we assessed gait stability and variability in a group of patients with focal cerebellar lesions after tumor resection. We specifically focused on stability in the medio-lateral direction in these mildly ataxic cerebellar patients. We quantified gait stability using the short-term maximum Lyapunov exponent (Bruijn et al., 2013; Dingwell and Cusumano, 2000) and we evaluated the margin of stability based on the extrapolated center of mass (Hof et al., 2005). We hypothesized that patients would walk with a less stable gait pattern and with a reduced margin of stability. Specifically, we expected that the short-term maximum Lyapunov exponent would make an important contribution to the description of gait deficits in this mildly ataxic patient group.

3.2 Materials and methods

3.2.1 Participants & Protocol

Eighteen cerebellar patients (age: 24.4 ± 7.3 yrs; $mean \pm SD$; 13 female, 5 male) and fourteen healthy participants (24.4 ± 3.5; 11 female, 3 male) participated in the study. All patients displayed chronic focal lesions after cerebellar tumor resection (various types, see Table 3.1). Nine patients received radiation therapy and four of them chemotherapy (Table 3.1). Lesion sizes are summarized in Table 3.1 (more details on lesion locations and on magnetic resonance imaging data acquisition and analysis procedures can be found in the supplementary materials). All patients were in a stable condition (> 2 years post-op; range $4.8 - 30.2$ yrs; Table 3.1). Severity of ataxia was rated using the International Cooperative Ataxia Rating Scale (ICARS; Trouillas et al., 1997). All participants gave written informed consent, as approved by the local ethics committee and in accordance with the Declaration of Helsinki.

Participants performed three trials of overground walking at self-selected speed, followed by three minutes of treadmill walking at 1.0 m/s. During treadmill walking 3D ground reaction forces were collected at 1,000 samples/s (custom built

Table 3.1: ***All patients had stable focal lesions after cerebellar tumor resection***

#	Age (years)	Time Post-op (years)	Sex	Diagnosis	Lesion Volume (cm^3)	Vermal Lesion Volume (cm^3)	ICARS Total /100	ICARS P&G /34	ICARS Kin Fun /52	Adjuvant Therapies Radiation	Adjuvant Therapies Chemo
1	28.8	13.9	f	Lhermitte Duclos Disease	58.0	-	3	1	1	-	-
2	20.2	8.7	f	Pilocytic Astrocytoma	8.2	-	3	0	0	-	-
3	18.1	6.5	f	Pilocytic Astrocytoma	4.5	-	1	0	1	-	-
4	19.6	11.8	m	Pilocytic Astrocytoma	1.7	1.1	6	3	2	-	-
5	20.5	4.8	f	Pilocytic Astrocytoma	47.3	5.6	3	1	1	-	-
6	20.5	13.1	f	Pilocytic Astrocytoma	36.3	-	2	1	0	-	-
7	19.0	13.2	m	Pilocytic Astrocytoma	15.7	-	7	2	5	-	-
8	41.2	28.1	f	Pilocytic Astrocytoma	20.2	4.9	20	6	10	Y	-
9	26.9	24.9	f	Pilocytic Astrocytoma	58.4	-	5	1	1	Y	-
10	22.0	18.7	m	Astrocytoma grade II	2.0	0.7	1	0	1	-	-
11	21.6	19.5	f	Astrocytoma grade II	7.1	2.1	0	0	0	Y	-
12	31.4	19.7	f	Astrocytoma grade III	8.6	0.4	11	5	4	Y	-
13	22.3	17.7	m	Medulloblastoma	22.0	3.3	13	6	2	Y	-
14	18.6	13.6	m	Medulloblastoma	6.3	2.5	19	5	10	Y	Y
15	18.4	15.5	f	Medulloblastoma	5.4	2.2	5	1	3	Y	Y
16	31.3	18.2	f	Medulloblastoma	14.2	4.0	17	10	4	Y	Y
17	18.5	10.0	f	Medulloblastoma	22.6	5.2	2	1	1	Y	Y
18	39.9	30.2	f	Hemangioblastoma	no MRI	no MRI	6	4	2	-	-

For patient 18 no MRI data was acquired. ICARS = International Cooperative Ataxia Rating Scale (Bruijn et al., 2009); P&G = Posture & Gait sub-score; Kin Fin = Kinetic Functions sub-score; f = female, m = male; Y = yes.

instrumented treadmill, Forcelink, Culemborg, The Netherlands). We recorded three-dimensional kinematics at 100 samples/s (Vicon Nexus, Oxford Metrics, Oxford, UK) using a marker cluster placed at the pelvis.

3.2.2 Data analysis

We calculated overground walking speed as the mean forward velocity of the pelvis marker cluster during the three overground walking trials. We calculated gait stability for the treadmill walking trials. Heel strike and toe-off events were extracted from the center of pressure trajectory (Roerdink et al., 2008). Gait parameters were based on 150 strides for each participant. Step width was defined as the medio-lateral distance between the average center of pressure locations during subsequent single stance phases. The coefficient of variance of stride time was calculated to assess stride time variability.

Gait stability was addressed by calculating the short-term maximum Lyapunov exponent (λ_S) from the medio-lateral displacement of pelvis marker cluster, following Bruijn's protocol (Bruijn et al., 2009). In short, the Euclidean distance between each data point in state space and its nearest neighbor was tracked over time. A divergence curve was constructed by taking the mean of the log of all these

time-distance curves. The short-term maximum Lyapunov exponent is the slope of this divergence curve over $0 - 0.5$ strides. Higher values for λ_S imply less gait stability (Dingwell and Cusumano, 2000; Bruijn et al., 2009).

Additionally, we calculated the 'margin of stability' between the 'extrapolated center of mass' and the medio-lateral base of support (Hof et al., 2005). To calculate the 'extrapolated center of mass' we estimated the center of mass and its velocity from the center of pressure trajectory. The margin of stability quantifies how close an inverted pendulum model of the participant would be from falling sideways. Therefore, a greater margin is associated to more stable gait.

3.2.3 Statistical analyses

We used Student's t-tests to compare gait parameters between groups. Correlations between specific gait parameters, ICARS (sub) scores and lesion measures were evaluated within the patient group using Pearson regression analysis.

3.3 Results

The cerebellar patients displayed relatively mild functional deficits ($ICARS = 6.9 \pm 6.4$, range $0 - 20$; Table 3.1) and self-selected a lower overground walking speed as compared to healthy controls (1.12 ± 0.12 m/s vs. 1.31 ± 0.17 m/s; $p = 0.001$; Table 3.2. When gait characteristics of both patients and healthy controls were compared at an equal speed of 1.0 m/s during treadmill walking, most gait parameters were similar (Table 3.2). However, the maximum short-term Lyapunov exponent was higher in the patient group; hence their gait pattern was less stable than that of the healthy controls. Mean step width was also significantly higher in the patient group (0.21 ± 0.03 m) than in the control group (0.19 ± 0.02 m; $p = 0.046$).

As group differences were observed for self-selected overground walking speed, λ_S, and step width, we evaluated how these measures correlated with clinical outcome measures as ICARS (sub) scores and (vermal) lesion sizes. From these measures only step width was significantly correlated to total ICARS score ($r = 0.57$; $p = 0.014$; Fig. 3.1). In addition, correlation between the Posture and Gait sub score and the Kinetic Function sub score on the one hand and step width on the other was comparable ($r = 0.53$ and $r = 0.54$, respectively). Furthermore, λ_S appeared correlated to Posture and Gait sub score, but this was just below significance level

Table 3.2: ***Cerebellar patients walk with reduced dynamic stability and wider steps***

	Patients Mean ± sd	Healthy Controls Mean ± sd	p-value
Self-selected overground walking speed [m/s]	1.12 ± 0.12	1.31 ± 0.17	**0.001**
Stride time [s]	1.11 ± 0.06	1.11 ± 0.05	0.91
Stance time [%]	64.72 ± 0.76	64.78 ± 0.73	0.83
Double support time [%]	15.06 ± 0.67	15.30 ± 0.67	0.31
Stride time variability [%]	2.56 ± 0.72	2.18 ± 0.67	0.14
Maximum Lyapunov exponent	1.72 ± 0.16	1.58 ± 0.14	**0.011**
Step width [m]	0.21 ± 0.03	0.19 ± 0.02	**0.046**
Margin of stability [mm]	82.6 ± 12.9	75.1 ± 10.4	0.08

($r = 0.46$; $p = 0.053$; Fig. 3.1). Finally, none of the parameters were significantly correlated to lesion size ($|r| < 0.4$; $p > 0.1$ for all), but within the patients with vermal lesions ($n = 11$; Table 3.1) the vermal lesion size was positively correlated to λ_S ($r = 0.64$; $p = 0.033$; Fig. 3.1). This indicates that the patients with the largest vermal lesions were the ones with the highest λ_S, i.e. lowest gait stability.

In order to address relations between different gait measures, we evaluated correlation between self-selected overground walking speed, stride time variability, λ_S, step width and margin of stability within the patient group. Margin of stability and step width were strongly correlated ($r = 0.96$; $p < 0.001$; Fig. 3.2). In addition, stride time variability was significantly correlated to λ_S ($r = 0.51$; $p = 0.031$; Fig. 3.2). None of the other correlations reached significance ($r = 0.44$; $p = 0.07$ for stride time variability vs. margin of stability; $|r| < 0.4$; $p > 0.1$ for all others).

3.4 Discussion

In this study we aimed to gain more insights into gait stability, gait variability and step width in patients with focal cerebellar lesions. Based on ICARS scores, the patients were only mildly ataxic, and most spatio-temporal gait parameters were similar between groups. When looking at more sensitive gait measures however, group differences could be observed. Gait stability was lower in the patient group (λ_S was higher), and correlated to vermal lesion size ($r = 0.64$; $p = 0.033$), indicating that the patients with the largest lesions in the vermis were the ones

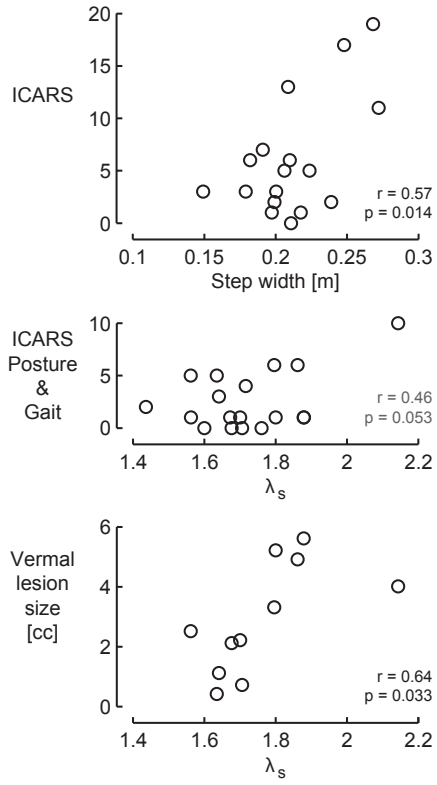


Figure 3.1: ***Step width and gait stability are correlated with clinical measures.*** ***A)*** *Step width was correlated to total ICARS score (International Cooperative Ataxia Rating Scale (Trouillas et al., 1997)).* ***B)*** *Correlation between the short-term maximum Lyapunov exponent (λ_S) and the ICARS sub score for Posture & Gait was not significant ($p = 0.053$).* ***C)*** *Short-term maximum Lyapunov exponent (λ_S) values for the patient subgroup with vermal lesions versus the size of the lesion. Vermal lesion size was positively correlated to λ_S, indicating that the patients with the largest vermal lesions were the ones with the lowest gait stability.*

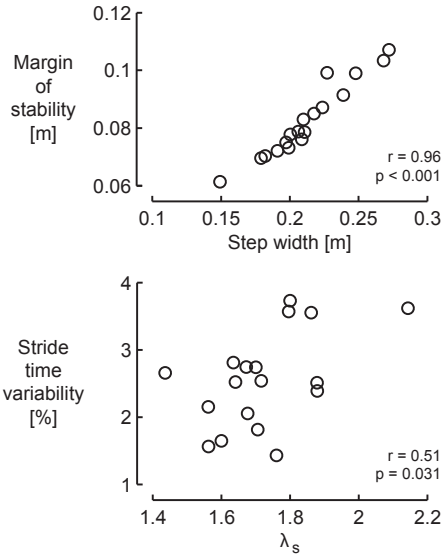


Figure 3.2: ***Several different gait parameters were correlated.*** ***A)*** *Margin of stability and step width were strongly correlated.* ***B)*** *Short-term maximum Lyapunov exponent (λ_S) was correlated to stride time variability.*

with the lowest gait stability. Such a role for vermal regions in gait stability is in line with what was hypothesized based on earlier observations of other gait measures as step width and stride-to-stride variations (Ilg et al., 2008, 2013). We did not apply more detailed lesion symptom mapping because our patient sample was rather heterogeneous with respect to either ataxia severity (range $0 - 20$) or radiation and chemotherapy history.

Interestingly, while gait stability and step width were different between these mildly ataxic patients and healthy controls, stride time variability was similar between groups. Hence, while on average this patient sample did not show significant deficits in gait variability, their gait stability was impaired. This suggests that, in these mildly ataxic patients, λ_S is a more sensitive measure of gait deficits than gait variability.

In addition to λ_S, we used the margin of stability to assess gait stability (Hof et al., 2005). While we expected to see a smaller margin of stability in the patient group, the margin of stability was actually similar between groups, with a tendency to be larger in the patients ($p = 0.08$). However, patients walked with wider steps than healthy controls. The fact that the margin of stability depends on the base

of support and the observation that the patient group walked with wider steps, suggest that step widening could be used as a strategy to ensure a sufficient margin of stability. This is supported by data on amputee gait, as amputees were observed to walk with wider steps than able-bodied controls, with a similar margin of stability (Curtze et al., 2011). Furthermore, during walking trials with continuous balance perturbations both amputees and able-bodied controls widen their steps in order to increase their margin of stability (Hak et al., 2013b). Also, when walking backwards, healthy subjects widen their steps and increase their margin of stability (Hoogkamer et al., 2012). Along this line of reasoning, we argue that the margin of stability and step width should not be used as mutually independent measures to classify gait as stable or instable, as both might or might not have been adapted in situations or populations where balance is challenged.

A next step to gain more insights into step widening as a compensation strategy could be to evaluate how the relation between step width and the margin of stability changes in cerebellar patients and healthy controls in conditions where normal gait is challenged. Furthermore, only patients in a stable recovery phase were included in the current study (time post-op $4.8 - 30.2$ yr; Table 3.1). Evaluating the margin of stability in patients in an initial recovery phase could reveal how the nervous system learns to compensate for specific cerebellar-related gait deficits. In addition, alternative compensation strategies should be evaluated in this mildly ataxic patient population as well, since Mari et al. (2014) recently identified antagonist muscle co-activation as a potential compensation strategy to reduce imbalance in inherited cerebellar ataxia patients.

In summary, we observed that patients with focal lesions in the cerebellum walked with lower gait stability and wider steps than healthy controls, while other gait parameters were similar. Patients with the largest vermal lesions displayed the least stable gait pattern. These observations in mildly ataxic patients confirm the importance of the cerebellum (and the vermis in particular) in the supraspinal control of gait in humans, specifically in relation to gait stability.

3.5 Acknowledgements

This work was supported by Research Foundation-Flanders (FWO grants G.0756.10 and G.0901.11) and the Netherlands Organisation for Scientific Research (NWO #451-12-041).

3.6 Supplementary materials

A Philips 3T Achieva MRI scanner (Philips, Best, The Netherlands) with a 32-channel matrix head coil was used for image acquisition. A 3D MPRAGE high resolution T1-weighted image (repetition time = 970 ms, echo time = 4.60 ms, flip angle = 8°, 230 1-mm slices, in-plane resolution = 0.97×0.98, 384×384 matrix) was acquired for all patients, except P18 (Table 3.3).

MRIcroN software (http://www.mccauslandcenter.sc.edu/mricro/mricron/index.html) was used to manually trace the lesions on the MPRAGE images. The SUIT toolbox (http://www.icn.ucl.ac.uk/motorcontrol/imaging/suit.htm Diedrichsen, 2006; Diedrichsen et al., 2011) in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) was used to spatially normalize the lesion traces to the atlas of the cerebellum (Diedrichsen et al., 2009). In some cases (large lesions at the outer border of the cerebellum) spatial normalization with the SUIT toolbox was inaccurate. In those cases lesions were spatially normalized based on the whole brain image and the normalized lesions were manually corrected in atlas space when needed, based on the original image. An overlap image of the normalized lesions is presented in Fig. 3.3, an overview of the lesioned lobules of each of the patients is provided in Table 3.3.

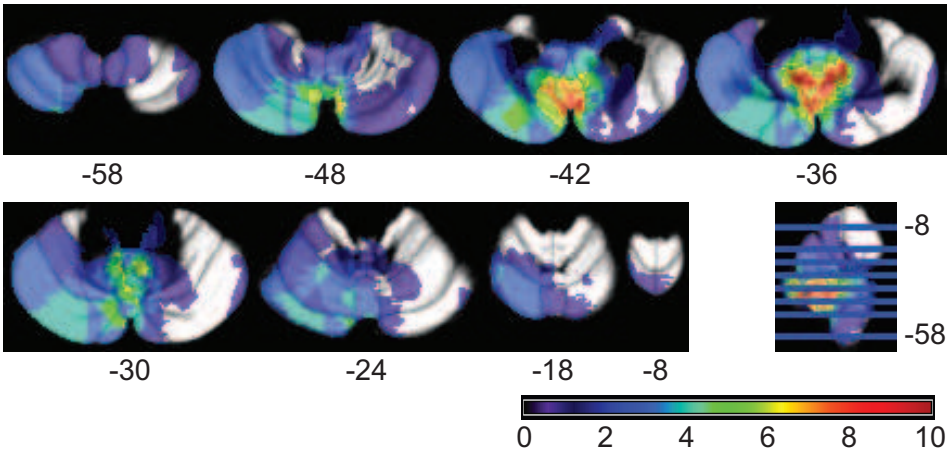


Figure 3.3: ***Overlap of lesions normalized to the SUIT template.*** *Lesion overlap is displayed on 8 axial slices as indicated in the sagital overview slice (bottom right panel). The number of overlapping lesions is indicated by colour code. Maximum overlap (10 patients) was around the border between the left paravermal lobules VIIb and Crus II (color coding according to the heat index below the cerebellar slices).*

Table 3.3: ***Overview of lesioned lobules***

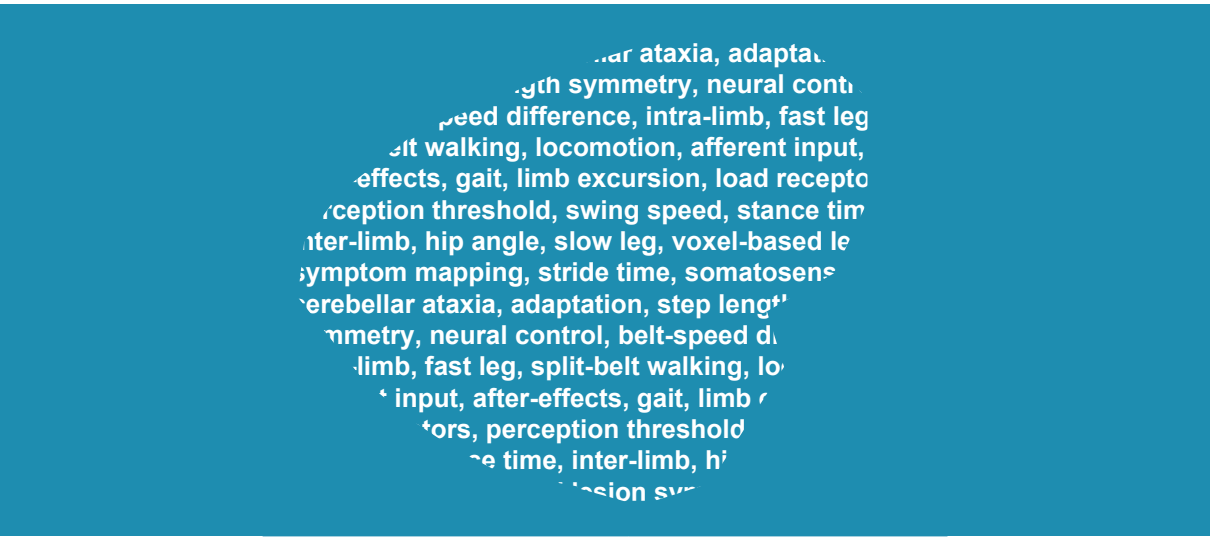
	Vermis								Paravermis									
#	VI	CI	CII	VIIb	VIIIa	VIIIb	IX	X	I-IV	V	VI	CI	CII	VIIb	VIIIa	VIIIb	IX	X
1									L	L	L	L	L	L	L	L		
2																		
3			Y	Y	Y	Y	Y						L	L	L	L	L	
4															R	R	R	R
5					Y	Y	Y	Y	L	B			B	B	B	B	B	
6																		
7										R	R	R	R	R	R		R	
8	Y	Y	Y	Y	Y	Y	Y				B	B	B	B	B	B	B	
9											L	L	L	L	L			
10	Y	Y	Y	Y	Y	Y							B	R				
11	Y		Y	Y	Y	Y	Y				R	R	R	B	R	R	R	
12					Y	Y	Y	Y						R	R	R	B	R
13					Y	Y	Y	Y	L	B			B	B	B	B	B	
14					Y	Y	Y	Y					R	R	R	R	B	
15	Y		Y	Y	Y	Y	Y	Y					B	B			B	
16	Y		Y	Y	Y	Y	Y	Y	B	B			B	B	B	B	B	
17	Y	Y	Y	Y	Y	Y	Y		B	B	L	L	B	B	B	B	B	
18				no MRI										no MRI				

	Lesion Volume	Hemispheres									Nuclei		
#	(cm3)	I-IV	V	VI	CI	CII	VIIb	VIIIa	VIIIb	X	FN	IN	DN
1	58.0	L	L	L	L	L	L	L	L				
2	8.2				R	R	R	R	R				
3	4.5											L	
4	1.7								R	R			
5	47.3					L	L				B	B	B
6	36.3			L	L	L	L	L					
7	15.7			R	R	R	R	R				R	R
8	20.2										B	B	
9	58.4			L	L	L	L	L					
10	2.0												
11	7.1											R	B
12	8.6										R	R	
13	22.0					L	L				B	B	B
14	6.3											R	B
15	5.4										R	B	B
16	14.2										B	B	B
17	22.6		L	L							B	L	B
18	no MRI				no MRI							no MRI	

Y = yes; L = left; R = right; B = both; FN = fastigial nuclei; IN = interposed nuclei; DN = dentate nuclei.

Part II

Split-belt adaptation, somatosensory perception and the cerebellum



4

Split-belt walking in cerebellar lesion patients I: Adaptation and after-effects

Submitted to Journal of Neurophysiology:
Hoogkamer W, Bruijn SM, Sunaert S, Swinnen SP, Van Calenbergh F, Duysens J. Split-belt walking in cerebellar lesion patients I: Adaptation and after-effects.

Abstract

To walk efficiently and stably on different surfaces and in different conditions, humans need to substantially adapt their gait pattern. Although the mechanisms behind locomotor adaptation are still not fully understood, the cerebellum is thought to play an important role. Here we aimed to address the specific localization of the cerebellar involvement in split-belt adaptation, by comparing performance in patients with stable focal lesions after cerebellar tumor resection and in healthy controls. We observed that changes in symmetry of inter-limb parameters, such as step length and relative double stance time during and after split-belt walking were similar between healthy controls and cerebellar patients. In contrast, relative stance times were more asymmetric for the patient group than for the control group during the early phase of the post- split-belt condition. Patients who walked with more asymmetric relative stance times were more likely to demonstrate lesions in vermal lobules VI and Crus II. These results confirm that deficits in gait adaptation vary with ataxia severity and between patients with different types of cerebellar damage.

Keywords

Ataxia; cerebellum; gait; locomotion; step length symmetry

4.1 Introduction

To walk efficiently and stably on different surfaces and in different contextual conditions, humans are able to substantially adapt their gait pattern. This locomotor adaptation is an important aspect of human gait and is often studied, both for its role in the neural control of gait (e.g. Choi and Bastian, 2007; Dietz et al., 1994) and for its role in motor learning and gait retraining (e.g. Lauzière et al., 2014a; Reisman et al., 2007, 2013). Although the mechanisms behind locomotor adaptation are still not fully understood, the cerebellum is thought to play an important role (Hoffland et al., 2014; Ilg et al., 2008; Jayaram et al., 2011, 2012; Morton and Bastian, 2006), similar to its involvement in visuomotor and force-field adaptations in the upper limbs (Burciu et al., 2014; Martin et al., 1996; Smith and Shadmehr, 2005; Tseng et al., 2007).

Locomotor adaptation is often studied using a split-belt paradigm (for review: Torres-Oviedo et al., 2011). In such a paradigm, participants walk on a dual-belt treadmill that consists of two parallel belts, one for each leg, which can run at different velocities (split-belt condition). In a classic split-belt adaptation paradigm, participants walk with one of the belts running at twice (or thrice) the speed of the other, for an extended period of time (5 to 15 minutes). Initially, participants walk with asymmetric step lengths, taking longer steps with the leg on the slow belt. Over time they adapt their gait pattern towards more symmetric step lengths. This locomotor adaptation results in after-effects when belts are returned to equal speeds. In this post-adaptation condition healthy participants again initially walk with asymmetric step lengths, but now taking longer steps with the leg that has been on the fast belt during the split-belt condition. Within a few minutes these after-effects disappear and step lengths return to baseline (symmetric) values. Changes in temporal gait parameters and in step length during split-belt walking were first described by the group of Dietz et al. (1994) and by Reisman et al. (2005), respectively. Since then split-belt adaptation has been studied in different conditions and in multiple (patient) populations (for review: Torres-Oviedo et al., 2011).

In an influential study, Morton and Bastian (2006) were able to show that severely ataxic patients with degenerative cerebellar disease do not display the typical features of split-belt adaptation. While these patients were able to quickly change intra-limb gait parameters when exposed to split-belt walking, similar to healthy controls, they did not adapt inter-limb gait parameters, while healthy controls did (Morton and Bastian, 2006). Specifically, limb excursion and relative stance time (intra-limb parameters, coordinated within one leg) changed similarly in both

groups from baseline to the early phase of the split-belt condition but no significant after-effects were observed in either group. Note that limb excursion was referred to as 'stride length' by Morton and Bastian (2006). In contrast, inter-limb gait parameters (coordinated between two legs), such as step length and relative double stance time, differed between groups. In the healthy controls these parameters changed from the early phase to the late phase of the split-belt condition and displayed a significant after-effect. On the other hand, in the severely ataxic patient group, no changes during the split-belt condition or after-effects were observed (Morton and Bastian, 2006).

In addition to these observations of impaired split-belt adaptation in severely ataxic patients with degenerative cerebellar disease (Morton and Bastian, 2006), other studies using a variety of brain stimulation techniques also found relations between the cerebellum and split-belt adaptation (Jayaram et al., 2011, 2012). However, while cerebellar involvement in locomotor adaptation has been observed repeatedly, it is still under debate where exactly this involvement is localized within the cerebellum (Ilg et al., 2008; Morton and Bastian, 2006). Morton and Bastian (2006) could not directly address localization due to the diffuse nature of the cerebellar damage in their patient group, but suggested that the midline vermis and fastigial nuclei would be most important in split-belt adaptation. Alternatively, Ilg et al. (2008) observed that damage in the intermediate cerebellum, the interposed nuclei and adjacent dentate nuclei was related to impaired locomotor adaptation. It should be noted, however, that they used a different paradigm (adaptation to added mass at the legs). Hence the question remains whether these results can be extrapolated to split-belt walking.

The aim of the present study was to address the localization of the cerebellar involvement in split-belt adaptation, by evaluating split-belt adaptation in patients with stable focal lesions after cerebellar tumor resection. Based on the observations by Morton and Bastian (2006) and Ilg et al. (2008), we hypothesized that these patients would show several impairments during split-belt adaptation and that these impairments would be most pronounced in patients with lesions in the interposed nuclei. Specifically, with respect to inter-limb coordination, we expected that patients would walk with a larger asymmetry in step lengths during the early phase of the split-belt condition and that this asymmetry would still be present during the late phase of the split-belt condition. Furthermore, we predicted that due to this reduced adaptation to split-belt walking, these patients would walk with more symmetric step lengths during the early phase of the post condition. Finally, we hypothesized that patients with focal cerebellar lesions would show similar changes in intra-limb gait parameters as healthy controls during the split-belt paradigm: no changes from the early to the late phase of the split-belt condition and no

after-effects during the post condition.

4.2 Materials and Methods

4.2.1 Participants

Fifteen patients with stable focal lesions after cerebellar tumor resection (age: 23.0 ± 6.2 years ($mean \pm SD$); 10 females, 5 males; Table 4.1) and thirteen healthy participants (age: 25.3 ± 4.6 years; 9 females, 4 males) participated. All patients suffered from cerebellar tumors (pilocytic astrocytoma grade I ($n = 6$), astrocytoma grade II ($n = 2$), medulloblastoma ($n = 5$), Lhermitte Duclos Disease ($n = 1$) or hemangioblastoma ($n = 1$); Table 4.1). Seven patients received adjuvant radiotherapy, four of these patients received adjuvant chemotherapy (overview: Table 4.1; therapy details: Table 4.2). Extra-cerebellar damage, assessed on MRI images, was mainly limited to a residually enlarged supratentorial ventricular system or sequela due to a ventriculo-peritoneal shunt in the right frontal lobe in some patients (Table 4.2). Patients were in a stable condition (> 5 years post-op; range $5.7 - 30.2$ yrs; Table 4.1) and were able to walk independently. All patients were able to walk on the treadmill without holding the hand railing. We rated severity of ataxia using the International Cooperative Ataxia Rating Scale (ICARS) (Trouillas et al., 1997). In this 100 point scale, a score of 0 indicates no deficits and increasing scores indicate more or more severe ataxic deficits. ICARS scores in our patient group ranged from 0 to 19, with only three patients scoring higher than 10 (Table 4.1). All participants gave written informed consent. The experiments were conducted in accordance with the Declaration of Helsinki and were approved by the local ethics committee.

4.2.2 Experimental set-up and protocol

In general, procedures were similar to Bruijn et al. (2012c). On arrival of the participant in the lab, reflective markers were placed on the pelvis and lateral malleoli of the participants for movement registration with an optoelectronic system (Vicon Nexus, Oxford Metrics, Oxford, UK). Throughout all conditions, kinematics were sampled at 100 samples/s. In addition, during the walking trials on the treadmill, three-dimensional ground reaction forces and torques were sampled at 1,000 samples/s (instrumented dual-belt treadmill, custom built by Forcelink, Culemborg, The Netherlands).

Table 4.1: ***Patients were mildly ataxic and in a stable condition (> 5 years post-op)***

#	Age (years)	Time Post-op (years)	Sex	Diagnosis	Interposed Nuclei Lesioned	Adjuvant Therapies		Lesion Volume (cm^3)	ICARS Total /100	ICARS P&G /34	ICARS Kin Fun /52
						Radiation	Chemo				
1	28.8	13.9	f	Lhermitte Duclos Disease				58.0	3	1	1
2	20.2	8.7	f	Pilocytic Astrocytoma				8.2	3	0	0
3	19.6	11.8	m	Pilocytic Astrocytoma	Left			1.7	6	3	2
4	18.1	6.5	f	Pilocytic Astrocytoma				4.5	1	0	1
5	22.3	17.7	m	Medulloblastoma	Both	Y		22.0	13	6	2
6	18.5	10.0	f	Medulloblastoma	Left	Y	Y	22.6	2	1	1
7	18.6	13.7	m	Medulloblastoma	Right	Y	Y	6.3	19	5	10
8	18.4	15.5	f	Medulloblastoma	Both	Y	Y	5.4	5	1	3
9	39.9	30.2	f	Hemangioblastoma	no MRI			no MRI	6	4	2
10	20.5	13.1	f	Pilocytic Astrocytoma				36.3	2	1	0
11	19.0	5.7	m	Pilocytic Astrocytoma	Right			15.7	7	2	5
12	22.0	18.7	m	Astrocytoma grade II				2.0	1	0	1
13	21.6	19.5	f	Astrocytoma grade II	Right	Y		7.1	0	0	0
14	26.9	24.9	f	Pilocytic Astrocytoma		Y		58.4	5	1	1
15	31.3	18.2	f	Medulloblastoma	Both	Y	Y	14.2	17	10	4

For patient 9 no MRI data was acquired; f = female, m = male; Y = yes; P&G = Posture & Gait sub score; Kin Fin = Kinetic Functions sub score.

Table 4.2: ***Treatment details***

#	Diagnosis	Time Post RT (years)	Target Areas Dose RT (Gy)	Hypo-pituitarism	Time Post CT (years)	Total Duration CT (months)	Scheme CT	VP shunt	Extra-cerebellar sequela
1	Lhermitte Duclos Disease							Y	
2	Pilocytic Astrocytoma							Y	
3	Pilocytic Astrocytoma								
4	Pilocytic Astrocytoma								
5	Medulloblastoma	17.6	35.2 CSP + 10 SP + 20 FP	Y				Y	Y*
6	Medulloblastoma	9.9	35.2 CSP + 20 FP		8.7	12	HIT-2000	Y	Y**
7	Medulloblastoma	13.1	35.2 CSP + 10 SP + 20 FP	Y	13.0	8	HIT-91	Y	Y**
8	Medulloblastoma	15.2	35.2 CSP + 10 SP + 20 FP	Y	15.2	3	HIT-91		Y***
9	Hemangioblastoma							Y	
10	Pilocytic Astrocytoma								
11	Pilocytic Astrocytoma								
12	Astrocytoma grade II								
13	Astrocytoma grade II	19.4	50.4 FP						
14	Pilocytic Astrocytoma	24.7	60 FP						Y**
15	Medulloblastoma	18.1	35.2 CSP + 10 SP + 20 FP	Y	17.5	4	HIT-2000	Y	Y**,****

f = female; m = male; Y = yes; RT = radiotherapy; CT = chemotherapy; CSP = craniospinal; SP = spinal; FP = fossa posterior; VP = ventriculo-peritoneal; HIT-2000 = cisplatinum, vincristine, CCNU; HIT-91 = ifosfamide, etoposide (VP16), metotrexate, ara-C, cisplatinum.

* Thalamic cavernous angioma, asymptomatic;

** Hydrocephalus;

*** Cavernous angioma parietal white matter, asymptomatic; cavernous angioma intramedullary spinal cord, level D12, 1.8 × 2.6 mm, asymptomatic;

**** Ventriculo-cisternal shunt

Before the split-belt trials, we assessed comfortable overground walking speed. Participants walked a distance of 6 m at their natural pace (Abellan van Kan et al., 2009; Bruijn et al., 2012c). This was repeated three times and all walking trials were performed barefoot. Next, participants were familiarized to treadmill walking. During the treadmill trials participants wore a safety harness attached to the ceiling and they were not holding the hand railing. The split-belt paradigm started with 3 minutes walking with both belts at 1.0 m/s (Baseline). Then participants performed a classical split-belt paradigm, consisting of ten minutes of walking with one belt at 1.0 m/s and the other belt at 0.5 m/s, followed by five minutes with both belts at the same speed (e.g. Bruijn et al., 2012c; Choi and Bastian, 2007; Morton and Bastian, 2006). During the split-belt condition (SPLIT) patients walked with the most affected side on the fast belt and healthy controls walked with their non-dominant leg on the fast belt (Morton and Bastian, 2006). In the subsequent tied-belt condition (POST) both belts ran at 1.0 m/s (Bruijn et al., 2012c), close to their preferred overground walking speed, but different from the study by Morton and Bastian (2006), where after-effects were assessed at 0.5 m/s. Between conditions the treadmill was stopped for a maximum of 30 s, and during the start of all treadmill conditions the belts had an acceleration of 0.3 m/s^2 (Bruijn et al., 2012c).

4.2.3 Data analyses

Generally, data analysis procedures were similar to (Bruijn et al., 2012c). We calculated overground walking speed as the mean forward velocity of the two posterior pelvis markers during the three overground walking trials (Hoogkamer et al., chapter 3). We determined the instants of heel strike and toe-off based on the center of pressure trajectory (Roerdink et al., 2008). All gait parameters calculated for the leg that was on the fast belt during the SPLIT condition are referred to as 'fast' parameters, even for the Baseline and POST conditions (Reisman et al., 2005); similarly for the 'slow' leg. Gait parameters were calculated stride-by-stride. Step length was calculated as the anterior-posterior distance between the ankle markers at heel contact (Reisman et al., 2005); with $step\ length_{fast}$ at the heel contact of the fast leg. Our main objective was to evaluate adaptation and after-effects in step length symmetry (Choi et al., 2009):

$$\text{Step Length Symmetry} = \frac{step\ length_{fast} - step\ length_{slow}}{step\ length_{fast} + step\ length_{slow}}$$

Similar to Morton and Bastian (2006) we also evaluated changes in symmetry of double support timing, limb excursion and stance time. Double Stance Symmetry was calculated similar to Step Length Symmetry, but based on the relative duration

of the double stance phase. The relative duration of the double stance phase occurring at the end of the stance phase of the fast leg was referred to as *double stance*$_{fast}$ (Reisman et al., 2005).

Limb excursion was calculated as the distance travelled by the ankle marker in the anterior-posterior direction from heel contact to toe-off of one limb (Hoogkamer et al., 2014b). Limb Excursion Symmetry was calculated analogue to Step Length Symmetry. Finally, Stance Time Symmetry was also determined in the same way, using the relative stance time of each leg. In other words, the proportion of stance in the gait cycle was compared for both sides.

Baseline values were calculated over all strides of the baseline condition. For statistical analyses we calculated values over the early and late phases of the SPLIT and POST conditions. Early SPLIT and Early POST values were calculated as the mean value over the first five strides of the respective conditions. Late SPLIT and Late POST values were calculated as the mean value over the last fifty strides of the respective conditions, to obtain a more accurate plateau value.

4.2.4 MRI data acquisition and processing

Image acquisition and processing procedures were similar to Hoogkamer et al. (2014d). A Philips 3T Achieva MRI scanner (Philips, Best, The Netherlands) with a 32-channel matrix head coil was used for image acquisition. A 3D MPRAGE high resolution T1-weighted image (repetition time = 970 ms, echo time = 4.60 ms, flip angle = 8°, 230 1-mm slices, in-plane resolution = 0.97 × 0.98, 384 × 384 matrix) was acquired for all patients, except for P9 (Table 4.1).

MRIcroN software (http://www.mccauslandcenter.sc.edu/mricro/mricron/index.html) was used to manually trace the lesions on the MPRAGE images. Lesion traces were spatially normalized to the atlas of the cerebellum (Diedrichsen et al., 2009, 2011) using the SUIT toolbox (http://www.icn.ucl.ac.uk/motorcontrol/imaging/suit.htm; Diedrichsen (2006)) in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). In some cases (large lesions at the outer border of the cerebellum) spatial normalization with the SUIT toolbox was inaccurate. In those cases lesions were spatially normalized based on the whole brain image and the normalized lesions were manually corrected in atlas space when needed, based on the original image (Ilg et al., 2008). Lobules and nuclei with lesions were listed (Table 4.3) and patients with lesions in the interposed nuclei were identified (Table 4.1). For further analysis, all left-sided normalized lesions were flipped to the right along the midline (Ilg et al., 2013).

Table 4.3: ***Overview of lesioned lobules and nuclei***

	Vermis								Paravermis									
#	VI	CI	CII	VIIb	VIIIa	VIIIb	IX	X	I-IV	V	VI	CI	CII	VIIb	VIIIa	VIIIb	IX	X
1									L	L	L	L	L	L	L	L		
2																		
3			Y	Y	Y	Y	Y						L	L	L	L	L	
4															R	R	R	R
5					Y	Y	Y	Y	L	B			B	B	B	B	B	
6	Y	Y	Y	Y	Y	Y	Y		B	B	L	L	B	B	B	B	B	
7					Y	Y	Y	Y					R	R	R	R	B	
8	Y		Y	Y	Y	Y	Y	Y					B	B			B	
9					no MRI									no MRI				
10																		
11										R	R	R	R	R	R		R	
12	Y	Y	Y	Y	Y	Y							B	R				
13	Y		Y	Y	Y	Y	Y				R	R	R	B	R	R	R	
14											L	L	L	L	L			
15	Y		Y	Y	Y	Y	Y	Y	B	B			B	B	B	B	B	

	Hemispheres									Nuclei		
#	I-IV	V	VI	CI	CII	VIIb	VIIIa	VIIIb	X	F	I	D
1	L	L	L	L	L	L	L	L				
2				R	R	R	R	R				
3											L	
4								R	R			
5					L	L				B	B	B
6		L	L							B	L	B
7											R	B
8										R	B	B
9					no MRI						no MRI	
10			L	L	L	L	L					
11			R	R	R	R	R				R	R
12												
13											R	B
14			L	L	L	L	L					
15										B	B	B

For patient 9 no MRI data was acquired; Y = yes; L = left; R = right; B = both; F = fastigial nuclei; I = interposed nuclei; D = dentate nuclei.

We hypothesized that patients with lesions in the interposed nuclei would show several impairments during split-belt adaptation: a reduced Step Length Symmetry during Early SPLIT and Late SPLIT and a more symmetric step length during Early POST, as compared to the healthy controls. To test this, we performed statistics on these outcome measures for the patient subgroups with and without lesions in the interposed nuclei (see Statistical analyses below).

In addition, for voxel-based lesion-symptom mapping the patients were classified as 'affected' or 'unaffected' based on behavioral outcome measures and then lesion locations between these subgroups were compared. This classification is commonly done with a cut-off threshold based on the behavioral data of the healthy controls and this is not always straightforward (Hoogkamer and Meyns, 2014). We applied a cut-off based on the 95%-confidence interval of the values for the healthy controls. This was done for Step Length Symmetry and in addition for Stance Time

Symmetry during Early POST. We used nonparametric mapping software within MRIcroN (http://www.mccauslandcenter.sc.edu/mricro/npm/; Rorden et al., 2007) to perform the voxel-based lesion-symptom mapping analysis. We applied both statistical Liebermeister tests and subtraction analysis to identify lesion areas associated with deviant behavior (Christensen et al., 2014). For the Liebermeister test, significance threshold was set to $Z = 1.65$ ($\alpha = 0.05$) and only voxels damaged in at least two patients were considered (Ilg et al., 2013). Subtraction analysis was performed by subtracting the percentage of normally performing patients with a lesion in a specific voxel from the percentage of patients with deviant behavior with a lesion in that voxel (Christensen et al., 2014; Karnath et al., 2002). This was done for each lesioned voxel. We considered voxels that were at least 25% more likely to be lesioned in patients with deviant behavior (Christensen et al., 2014).

4.2.5 Statistical analyses of behavioral data

Student's t-test was used to compare overground walking speed and global gait parameters during Baseline between groups. To evaluate changes over time and differences between groups in gait parameters during the split-belt paradigm, we performed two-factor repeated-measures ANOVAs with group and episode as factors. For each gait parameter we performed an ANOVA to compare Early SPLIT and Late SPLIT with baseline values. An additional ANOVA was performed to compare Early POST and Late POST with baseline values. To compare gait parameters between the subgroups with and without lesions in the interposed nuclei (see above) and the healthy control group, we performed a one-way ANOVA with three groups. For significant main and interaction effects we performed Tukey's honestly significant difference post-hoc analyses to identify significant differences between groups and/or episodes. We used a traditional level of significance ($\alpha = 0.05$) for all statistical tests; when appropriate, this value was corrected for the number of analyses.

4.3 Results

Self-selected overground walking speed was reduced in patients with cerebellar lesions as compared to healthy controls (1.13 ± 0.13 m/s vs. 1.35 ± 0.13 m/s, respectively; $p < 0.001$). During treadmill walking in the baseline condition (1.0 m/s, tied belts), gait parameters were similar between groups: stride time was 1.12 ± 0.06 s vs. 1.12 ± 0.06 s ($p = 0.90$), stride time variability (SD) was

28 ± 8 ms vs. 28 ± 14 ms ($p = 0.99$), step length was 0.51 ± 0.03 m vs. 0.51 ± 0.02 m ($p = 0.54$) and the relative stance time was $64.9 \pm 0.7\%$ vs. $65.0 \pm 0.5\%$ ($p = 0.57$) for the cerebellar lesion patients and the healthy controls, respectively. As such, these gait parameters confirmed that this group of patients with focal lesions in the cerebellum was only mildly ataxic (see Table 4.1 for results of clinical evaluation).

During the split-belt condition and the subsequent tied-belt condition, patients and healthy controls globally displayed similar changes in gait parameters. However, for some parameters important differences between the two groups were observed and these will be described in detail below.

4.3.1 Inter-limb parameters

First, we evaluated the parameters that were expected to differ, namely the inter-limb gait parameters: Step Length Symmetry and Double Stance Symmetry. However, in both groups, these parameters changed similarly during the split-belt paradigm (Fig. 4.1). There was asymmetry during the early phase of the SPLIT condition and values approached to symmetry in the late phase of the SPLIT condition. The curve for Step Length Symmetry suggests that the patients return to more symmetric values in Late SPLIT, however there was no significant main effect for group ($p = 0.45$; Table 4.4) or group×episode interaction effect (Fig. 4.1 A; $p = 0.81$). Double Stance Symmetry had not completely returned to baseline values during Late Split ($p = 0.024$). During the POST condition both Step Length Symmetry and Double Stance Symmetry initially show an overshoot in asymmetry and gradually return to symmetric values.

4.3.2 Intra-limb parameters

Secondly, we evaluated the parameters that were not expected to differ between groups, namely the intra-limb gait parameters: Limb Excursion Symmetry and Stance Time Symmetry. Both these parameters changed significantly during SPLIT (Fig. 4.2; Table 4.4). The positive Limb Excursion Symmetry values indicate that limb excursion of the fast leg was higher than that of the slow leg. The negative Stance Time Symmetry values indicate that relative stance times of the fast leg were shorter than those of the slow leg. No significant main effects for group or group×episode interaction effect was observed for Limb Excursion Symmetry and Stance Time Symmetry during SPLIT. During POST, a significant asymmetric overshoot was observed during Early POST, returning to baseline values in Late POST, for both parameters. The asymmetry in relative stance times during Early

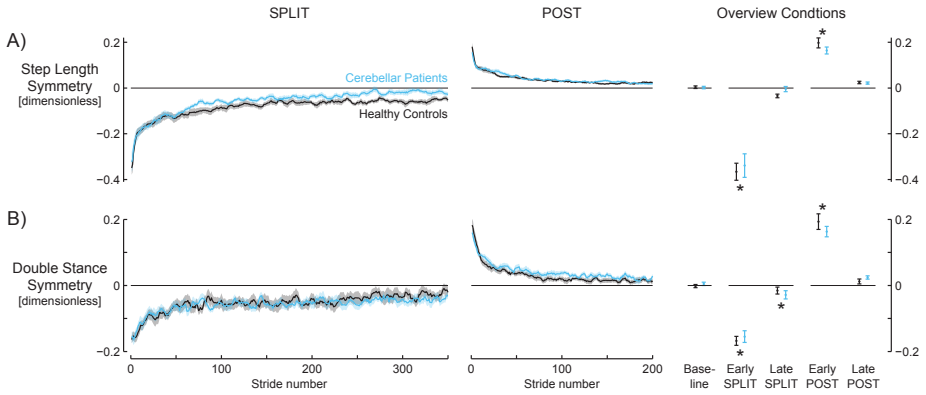


Figure 4.1: ***Inter-limb gait parameters show similar changes for cerebellar lesion patients and healthy controls during split-belt walking. A)*** *Step Length Symmetry,* ***B)*** *Double Stance Symmetry. Traces for cerebellar patients (blue) and healthy controls (black) during SPLIT (left panel) and POST (middle panel). Right panels summarize values during the different phases and conditions. Shaded areas and error bars represent ±1 SEM; * indicates values significantly different from baseline.*

4

POST was larger for the patient group (0.04 ± 0.03) than for the control group (0.00 ± 0.02; $p = 0.001$).

4.3.3 Individual limbs

To further address the group difference in Stance Time Symmetry, we evaluated the relative stance times of the individual limbs (Fig. 4.3 A). During SPLIT the between limb difference in relative stance times became smaller. This occurred simultaneous with both an increase in the relative stance time of the fast leg ($p = 0.040$) and a decrease in the relative stance time of the slow leg ($p < 0.001$) from Early to Late SPLIT. During Early POST, the increased asymmetry in relative stance times was related to both an increased relative stance time of the fast leg compared to Baseline ($p < 0.001$) and a decreased relative stance time of the slow leg compared to Baseline ($p = 0.019$). During Early POST the relative stance times were more asymmetric for the patient group than for the control group. When the fast and slow leg were evaluated separately, the group×episode interaction effect was only significant for the slow leg ($p = 0.017$), not for the fast leg ($p = 0.20$). Post-hoc analysis revealed that the relative stance time of the slow leg was lower in the

Table 4.4: ***Summary of statistics***

	SPLIT						POST					
	ANOVA		Post-hoc				ANOVA		Post-hoc			
Parameter	factor	p-value		values	comparison	p-value	factor	p-value		values	comparison	p-value
Step	group	0.45					group	0.27				
Length	episode	**<0.001**	BL	0.00±0.02	BL vs ES	**<0.001**	episode	**<0.001**	BL	0.00±0.02	BL vs EP	**<0.001**
Symmetry			ES	-0.35±0.17	ES vs LS	**<0.001**			ES	0.18±0.07	EP vs LP	**<0.001**
			LS	-0.02±0.04	BL vs LS	0.70			LS	0.02±0.02	BL vs LP	0.14
	interaction	0.81					interaction	0.22				
Double	group	0.87					group	0.75				
Stance	episode	**<0.001**	BL	0.00±0.02	BL vs ES	**<0.001**	episode	**<0.001**	BL	0.00±0.02	BL vs EP	**<0.001**
Symmetry			ES	-0.16±0.06	ES vs LS	**<0.001**			ES	0.18±0.07	EP vs LP	**<0.001**
			LS	-0.02±0.04	BL vs LS	**0.024**			LS	0.02±0.02	BL vs LP	0.27
	interaction	0.32					interaction	0.11				
Limb	group	0.93					group	0.33				
Excursion	episode	**<0.001**	BL	-0.00±0.01	BL vs ES	**<0.001**	episode	**<0.001**	BL	-0.00±0.01	BL vs EP	**<0.001**
Symmetry			ES	0.16±0.06	ES vs LS	**<0.001**			ES	0.03±0.03	EP vs LP	**<0.001**
			LS	0.23±0.03	BL vs LS	**<0.001**			LS	0.00±0.01	BL vs LP	0.74
	interaction	0.99					interaction	0.07				
Stance	group	0.43					group	**0.009**	HC	0.00±0.00		
Time									P	0.02±0.00		
Symmetry	episode	**<0.001**	BL	0.00±0.01	BL vs ES	**<0.001**	episode	**<0.001**	BL	0.00±0.01	BL vs EP	**<0.001**
			ES	-0.11±0.04	ES vs LS	**<0.001**			ES	0.02±0.03	EP vs LP	**<0.001**
			LS	-0.07±0.04	BL vs LS	**<0.001**			LS	0.00±0.01	BL vs LP	0.59
	interaction	0.99					interaction	**0.012**	HC	-0.00±0.01	HC vs P	0.98
									P	0.00±0.01		
									HC	0.00±0.02	HC vs P	**0.001**
									P	0.04±0.03		
									HC	0.00±0.00	HC vs P	0.96
									P	0.01±0.01		
Stride	group	0.45					group	0.23				
Time	episode	**<0.001**	BL	1.12±0.06	BL vs ES	1	episode	**<0.001**	BL	1.12±0.06	BL vs EP	**<0.001**
			ES	1.12±0.17	ES vs LS	**<0.001**			ES	1.03±0.12	EP vs LP	**<0.001**
			LS	1.30±0.15	BL vs LS	**<0.001**			LS	1.13±0.07	BL vs LP	0.62
	interaction	0.39					interaction	**0.001**	HC	1.12±0.06	HC vs P	1
									P	1.12±0.06		
									HC	0.98±0.14	HC vs P	**0.041**
									P	1.07±0.09		
									HC	1.13±0.04	HC vs P	1
									P	1.13±0.07		

BL = Baseline; ES = Early SPLIT; LS = Late SPLIT.

patient group ($62.7 \pm 3.2\%$) than in the control group ($65.3 \pm 2.3\%$; $p = 0.004$). Furthermore, during Early POST, the relative stance times of both legs were significantly correlated to the Stance Time Symmetry (fast leg: $r = 0.67$; $p < 0.001$; Fig. 4.3 B; slow leg: $r = -0.89$; $p < 0.001$; Fig. 4.3 C). Multiple regression analysis revealed that the relative stance time of the slow leg explained a larger part (55%) of the variance in Stance Time Symmetry than the relative stance time of the fast leg (21%).

4.3.4 Stride times

Along with the changes in the inter-limb and intra-limb gait parameters, changes in the stride time were observed (Fig. 4.3 D; Table 4.4). Stride time increased from Early SPLIT to Late SPLIT and was reduced during Early POST compared to

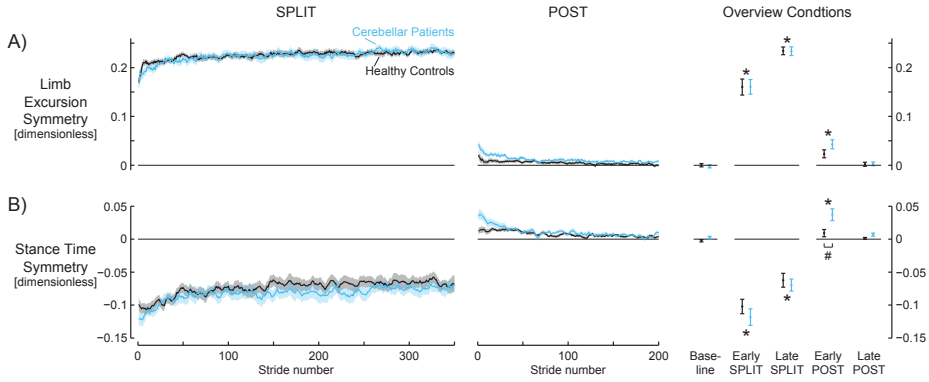


Figure 4.2: ***Intra-limb parameters displayed adaptation and after-effects for both cerebellar lesion patients and healthy controls during split-belt walking. A)*** *Limb Excursion Symmetry,* ***B)*** *Stance Time Symmetry. Traces for cerebellar patients (blue) and healthy controls (black) during SPLIT (left panel) and POST (middle panel). Right panels summarize values during the different phases and conditions. Shaded areas and error bars represent* ± 1 *SEM; * indicates values significantly different from baseline; # indicates values significantly different between groups.*

during Baseline. While stride time was similar between groups at Baseline and during Late Post, during Early Post healthy controls walked with shorter stride times (0.98 ± 0.14 s) than the patient group (1.07 ± 0.09 s; $p = 0.041$). To evaluate whether during Early POST the group difference in Stance Time Symmetry was related to the group difference in stride time a regression analysis was performed. This showed indeed a significant correlation between stride time and Stance Time Symmetry ($r = 0.47$; $p = 0.011$; Fig. 4.3 B).

4.3.5 Lesion mapping of locomotor adaptation

Eight patients had lesions in the interposed nuclei, six patients had no lesions in the interposed nuclei and one patient was not included in this analysis because no MRI data was available (Table 4.1). During Early SPLIT no significant group effect on step length symmetry was observed ($p = 0.95$; Fig. 4.4 A). During Late SPLIT a significant group effect was observed ($p = 0.046$, Fig. 4.4 B). Post-hoc inter-group comparisons suggest that the healthy controls had less symmetric step lengths than both patients with and patients without lesions in the interposed nuclei, but these

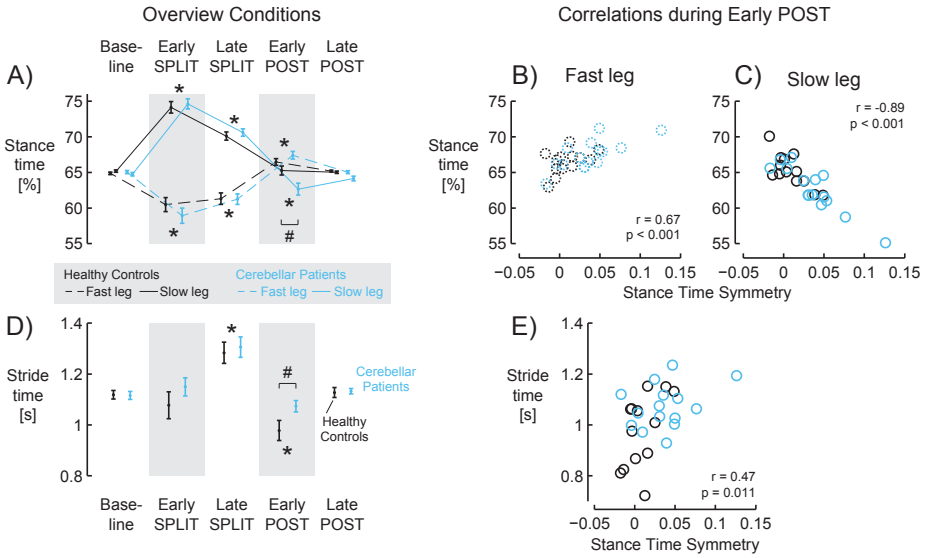


Figure 4.3: ***Changes in relative stance times and stride time during split-belt walking (A & D) and correlations with stance time symmetry during the early phase of the POST condition (B, C & E).*** ***A)*** *Relative stance times of the fast and the slow leg changed in opposite directions during split-belt walking. After-effects (Early POST) were significantly different from baseline and for the slow leg this after effect was larger in the patient group.* ***B) & C)*** *Relative stances times of both legs were correlated with Stance Time Symmetry during the early phase of the POST condition.* ***D)*** *Stride time was increased during Late SPLIT and reduced during Early POST; this reduction was most pronounced for the healthy controls.* ***E)*** *Stride time and Stance Time Symmetry were correlated during the early phase of the POST condition. Values for cerebellar patients are presented in blue and for healthy controls in black. Values for the fast leg are presented with dashed lines and for the slow leg with full lines. Error bars represent* ± 1 *SEM; * indicates values significantly different from baseline; # indicates values significantly different between groups.*

differences were not significant ($p = 0.081$ and $p = 0.118$, respectively). Finally, during Early POST no significant group effect was observed ($p = 0.52$; Fig. 4.4 C).

To further explore potential relations between focal cerebellar damage and split-belt walking behavior, we applied voxel-based lesion-symptom mapping (see lesion overlap in Fig. 4.5 A; Table 4.3). We evaluated both the Step Length Symmetry during the episodes mentioned above and the Stance Time Symmetry, which was observed to be different between groups during Early POST.

With regard to Step Length Symmetry during SPLIT and POST, patients were classified 'affected' when symmetry values were below the 95%-confidence interval of the values for the healthy controls (Fig. 4.4 A, B, C). Only one patient (P5; Table 4.1) could be classified to have a reduced Step Length Symmetry during Early SPLIT (Fig. 4.4 A); during the other episodes none of the patients displayed values lower than the cut-off threshold (Fig. 4.4 B, C). Therefore, voxel-based lesion-symptom mapping based on Step Length Symmetry was not feasible. The disproportionately reduced Step Length Symmetry during Early SPLIT for P5 (Fig. 4.4 A) was related to a negative step length during one of the first strides, where the foot of fast leg was placed posterior from the slow leg's foot. This also occurred for the single healthy control who's Step Length Symmetry was lower than the cut-off threshold (Fig. 4.4 A).

Stance Time Symmetry during Early POST was higher in the patient group than in the control group (see above) and therefore patients were classified 'affected' with values above the 95%-confidence interval of the values for the healthy controls (Fig. 4.4 D). Five patients had larger differences in relative stance times than the cut-off threshold (P4, P6, P8, P10, P12; Table 4.1). Subtraction analysis and the statistical Liebermeister test revealed importance for similar regions (Fig. 4.5 B, C). These were primarily in the posterior vermis; vermal lobules VI and Crus II, with, according the subtraction analysis, extensions into vermal lobules VIIb and VIIIa.

4.4 Discussion

The aim of the present study was to address the localization of the cerebellar involvement in split-belt adaptation. For this reason we included relatively mildly affected patients having reasonably well-localized lesions. Group differences were observed in Stance Time Symmetry during Early POST: relative stance times were more asymmetric for the patient group than for the control group. Patients who

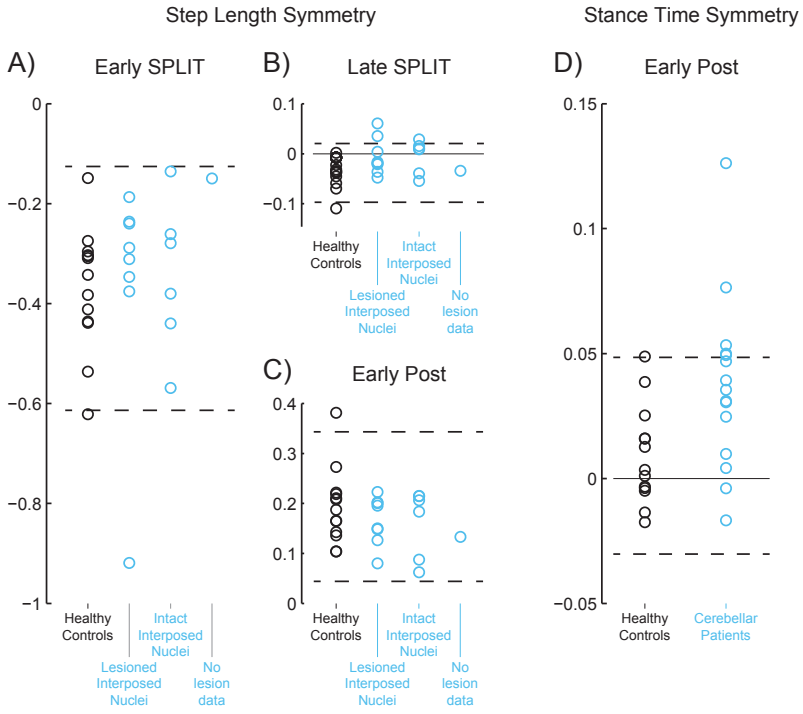


Figure 4.4: ***Lesions in the interposed nuclei were not related to differences in Step Length Symmetry; several patients walked with asymmetric stance times during the early phase of the POST condition.*** ***A)*** *Step Length Symmetry was similar between groups during Early SPLIT. One patient walked with more asymmetric step lengths: value below 95%-confidence interval of the values for the healthy controls (indicated by dashed lines).* ***B)*** *Step Length Symmetry was similar between groups during Late SPLIT. None of the patients walked with more asymmetric step lengths than the healthy controls.* ***C)*** *Step Length Symmetry was similar between groups during Early POST. All patient values are within the 95%-confidence interval of the values for the healthy controls.* ***D)*** *Five patients walked with increased asymmetry in relative stance times during Early POST: values above 95%-confidence interval of the values for the healthy controls. Values for cerebellar patients are presented in blue and for healthy controls in black; dashed lines indicate 95%-confidence interval of the values for the healthy controls.*

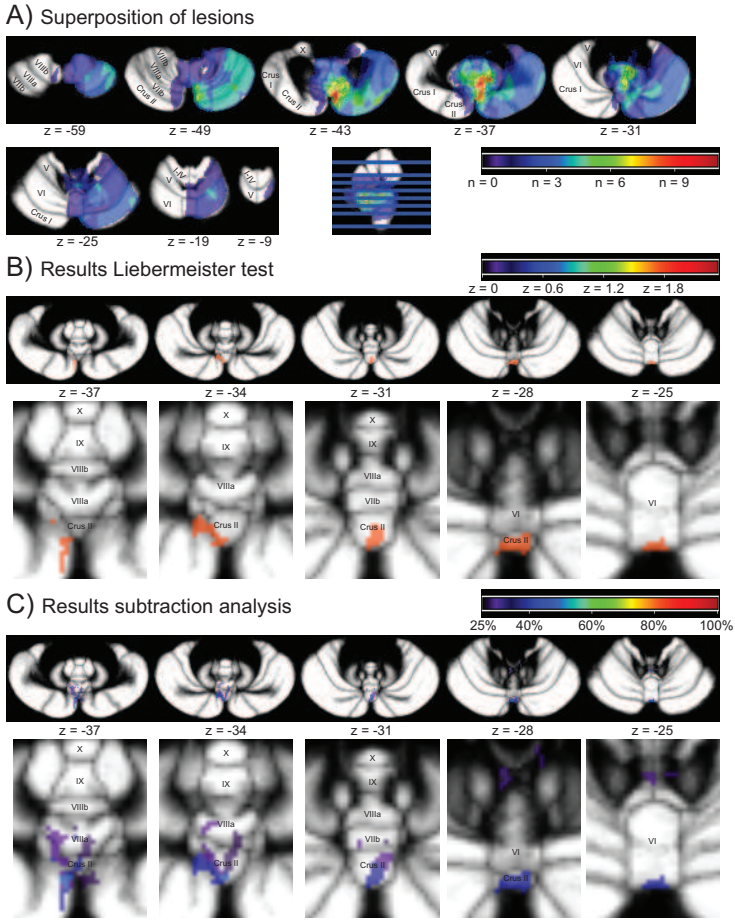


Figure 4.5: ***Lesion overlap and overview of areas related to an increased asymmetry in relative stance times during the early phase of the POST condition. A)*** *Superposition of the regions of cerebellar lesions of all patients. Note that lesions were flipped to the right for analysis. Maximum overlap (10 patients) was within vermal lobule VIIIa and paravermal lobules VIIb and Crus II (color coding according to the heat index above cerebellar slices).* ***B)*** *Vermal lobules VI and Crus II were significantly correlated to an increased asymmetry in relative stance times during Early POST. Regions with z-values* > 1.65 *(*$p < 0.05$*), resulting from the Liebermeister test are indicated (color coding according to the heat index above cerebellar slices).* ***C)*** *Subtraction analysis identified the same regions; regions that were at least 25% more likely to be lesioned in patients with increased asymmetry in relative stance times are indicated (color coding according heat index above cerebellar slices).*

walked with more asymmetric relative stance times were more likely to have lesions in vermal lobules VI and Crus II.

In general, the differences between patients and controls were small, in line with the mild degree of the deficits in the patient group. The present observation that patients with focal cerebellar lesions did not show any deficits in adaptation of Step Length Symmetry may appear to be in contrast with observations of such deficits in patients with diffuse cerebellar damage (Morton and Bastian, 2006). However, an important, often overlooked, element is that Morton and Bastian (2006) only included severely ataxic patients (ICARS > 30) in their main study. In additional analyses they showed that adaptation impairments were related to severity of (posture and gait) ataxia. Patients in our study had less severe ataxia, with ICARS scores ranging from 0 to 19 and only three patients scoring higher than 10. We did not observe a significant correlation between ICARS posture & gait sub-score and Step Length Symmetry during Early POST, but the patient with the highest sub-score (P&G = 10; P15; Table 4.1) did show the smallest asymmetry in step length of all participants. Furthermore, the patient that showed the lowest Step Length Symmetry during Early SPLIT (P5; Fig. 4.4 A) also had a rather high posture & gait sub-score (P&G = 6; Table 4.1) as compared to the other patients.

The mild degree of ataxia may also explain some of the differences observed with other studies. For example, we observed no differences in split-belt adaptation between patients with and patients without lesions in the interposed nuclei. This observation may appear in contrast with the observation that the interposed nuclei are important in adaptation of limb coordination, when walking with added mass at the shanks (Ilg et al., 2008). Again one could argue that such differences were related to differences in patients. However, interestingly, ataxia severity of the patients with focal lesions in the latter study was similar to that of our patients (Ilg et al., 2008). This could suggest that adaptation to split-belt walking and adaptation to added-mass walking are rather different processes. The latter is impaired in mildly ataxic patients with focal lesions, specifically in patients with lesions in the interposed nuclei. In contrast, adaptation of inter-limb parameters in split-belt walking was not observed to be impaired in mildly ataxic patients or to be dependent of the interposed nuclei. Split-belt adaptation appears to be more related to the control of posture and gait (Morton and Bastian, 2006) while added-mass adaptation appears to be more related to the control of multi-joint movements (Ilg et al., 2008).

4.4.1 Intra-limb parameters

While most recent split-belt studies have focused mainly on changes in inter-limb parameters such as step length, we observed significant after-effects in intra-limb parameters as well. These after-effects were most prominent in the patient group (Fig. 4.4) and were closely related to the relative stance time of the slow leg specifically. During the SPLIT condition the relative stance times of the slow leg were higher than those of the fast leg. During the SPLIT trial the participants reduced the relative stance time of the slow leg from the early to the late phase. In the patients, a larger part of this reduction was still present in the early phase of the POST condition (storage & transfer) as they walked with shorter relative stance times in the slow leg than the controls. While the larger after-effects in intra-limb parameters are not necessarily a deficit in adaptation, their presence suggests that the patients used a slightly different strategy to adapt their gait pattern to split-belt walking, reminiscent of the faster adjustments in swing times in elderly (Bruijn et al., 2012c).

Furthermore these data revealed some lesion site dependencies. We identified five patients with larger differences in relative stance times than the healthy controls (Fig. 4.4 D) and these patients were more likely to have lesions in the posterior vermis: vermal lobules VI and Crus II (Fig. 4.5 B, C). However, it should be noted that the identified regions were small and that accompanying Z-values ($z = 1.8$) and subtracted percentages were low ($< 50\%$). Traditionally these regions have not been endowed with an important motor control function, but several observations from lesion and functional MRI studies suggest otherwise. From structural and functional connectivity studies these regions appear to be mainly related to the limbic system (Stoodley and Schmahmann, 2010) and fronto-parietal and dorsal attention networks (Buckner et al., 2011). In lesion studies, vermal lobule Crus II has been related to visuomotor adaptation, both in cerebellar stroke patients (chronic) and in patients with cerebellar degeneration (Donchin et al., 2012). In patients with acute and subacute stroke lesions, this relation was not observed (Burciu et al., 2014). Studies on force field adaptation and added-mass walking did not observe a significant role for vermal lobules VI and Crus II (Burciu et al., 2014; Donchin et al., 2012; Ilg et al., 2008). It should be noted, however, that lesion overlap images from those studies suggest that very few patients had lesions in these regions. The posterior vermis has been related to performance on tandem walking (Bastian et al., 1998), but all lesions in that patient sample included vermal lobule X, which can be expected to be more important in relation to balance (Stoodley and Schmahmann, 2010). In functional MRI studies, vermal lobule CII has been related to eye-hand coordination motor learning (Miall and Jenkinson,

2005). Functional imaging studies from our group using bimanual coordination tasks have repeatedly observed involvement of cerebellar lobule VI, most often in the paravermal and hemispheric regions (Debaere et al., 2003; Heuninckx et al., 2005; Swinnen et al., 2010; Wenderoth et al., 2004, 2005), but also in vermal lobule VI (Beets et al., 2014; Debaere et al., 2004). Furthermore, both vermal lobules VI and Crus II have been observed to be enlarged in well-trained basketball players, based on MRI volumetry analyses (Park et al., 2009).

4.4.2 Limitations

Since our aim was to address the localization of the cerebellar involvement in split-belt adaptation, we included patients with focal cerebellar lesions. These patients are commonly only mildly ataxic, which turned out to be a limitation for our study, as argued above. Secondly, even though we aimed to include mainly patients who did not receive adjuvant radio- or chemotherapy, several patients had extra-cerebellar damage (Table 4.2). This is sub-optimal for lesion symptom mapping analysis (Timmann et al., 2009), but it did not bias our results: subtraction analysis within the subset of patients who did not receive adjuvant therapies, indicated importance for similar regions as were identified in our main analysis (vermal lobules VI and Crus II) and in addition for paravermal lobule IX. Another limitation of lesion analysis is that, while it gives indications of how behavior changes when a specific region is dysfunctional, it does not prove that this region is involved under healthy conditions. Furthermore, regions that are important for specific functions might not be identified, either because few if any of the patients have lesions in these regions or because other regions compensate for the deficits.

To further address the functional localization of locomotor adaptation, future studies could include patients with more severe forms of ataxia. Voxel-based morphometry could be used to localize deficits in cerebellar degeneration patients. Studies on severely ataxic patients with focal lesions (e.g. subacute or chronic stroke) could also provide useful insights. It should be mentioned however, that this group is not easy to obtain since stroke lesions are seldom limited to the cerebellum. Furthermore, stroke patients are often at advanced age which could confound behavioral outcomes (Bruijn et al., 2012c). Finally, it should be mentioned that when studying severely ataxic patients, there is also the possibility that behavioral outcomes are confounded by other deficits (in balance or muscle coordination) or by compensation strategies to cope with those.

In summary, we observed that changes in symmetry of inter-limb parameters,

such as step length and relative double stance time during the SPLIT and POST conditions were similar between healthy controls and mildly ataxic patients with focal cerebellar lesions. Relative stance times were more asymmetric for the patient group than for the control group during the early phase of the POST condition. Patients who walked with more asymmetric relative stance times were more likely to have lesions in vermal lobules VI and Crus II.

4.5 Acknowledgements

We thank Katrien Bastenie and Iris Degrande for assistance during the data collections and Inge Leunissen for fruitful discussions. This work was supported by Research Foundation-Flanders (FWO grants G.0756.10 and G.0901.11) and The Netherlands Organisation for Scientific Research (NWO grant #451-12-041). JD was supported by a visiting professorship from the National Research Council of Brazil (CNPq 478863/2010-1).

5

Split-belt walking in cerebellar lesion patients II: Role of somatosensory perception

Submitted to Journal of Neurophysiology:
Hoogkamer W, Bruijn SM, Potocanac Z, Van Calenbergh F, Swinnen SP, Duysens J. Split-belt walking in cerebellar lesion patients II: Role of somatosensory perception.

Abstract

Gait adaptation is essential for humans to be able to walk easily on different surfaces and in different conditions. Although locomotor adaptation has been studied in different contexts and in various patient populations, the mechanisms behind locomotor adaptation are still not fully understood. The aim of the present study was to test two opposing hypotheses about the control of split-belt walking, one based on avoidance of limping, the other on avoiding limb excursion asymmetry. We assessed how well participants (both cerebellar patients with focal lesions and healthy controls) could sense differences between belt speeds during split-belt treadmill walking and correlated this to split-belt adaptation parameters. We observed a significant inverse correlation between Stance Time Symmetry and Limb Excursion Symmetry during the early phase of split-belt walking. Participants who were better able to perceive belt speed differences walked with the smallest asymmetry in stance time and the largest asymmetry in limb excursion. Our data supports the hypothesis that humans aim to minimize (temporal) limping rather than (spatial) limb excursion asymmetry when using their perception of belt speed differences in the early phase of adaptation to split-belt walking.

Keywords

Ataxia; cerebellum; limping; locomotion; temporal gait symmetry

5.1 Introduction

Gait adaptation is a remarkable ability of many biological systems including humans with important implications for survival in complex environments. It has been studied extensively in the last years, either by using a dual-belt treadmill (using different speeds on each side; for review: Torres-Oviedo et al., 2011) or by using force-fields (e.g. Barthélemy et al., 2012; Blanchette and Bouyer, 2009; Ilg et al., 2008). In the earliest studies on split-belt walking of humans, it was already discovered that humans could easily adjust to walking with each leg at a different speed, with speed ratios up to 1 : 4 (Dietz et al., 1994). Moreover, it was found that when the belts were returned to equal speeds after a period of walking with different speeds (split-belt), after-effects were visible. These after-effects suggested that gait pattern adaptations made during the split-belt condition were stored, and thus feedforward controlled.

The time course of this adaptation process has been studied in detail in recent years (Bruijn et al., 2012c; Finley et al., 2014; Malone and Bastian, 2010; Tyrell et al., 2014; Vasudevan et al., 2011). Using a split-belt condition, the group of Bastian found that intra-limb parameters (such as limb excursion; previously termed stride length; Hoogkamer et al., 2014b) adjust rapidly, and thus can be considered as feedback controlled parameters (rather than feedforward; Morton and Bastian, 2006; Reisman et al., 2005). In contrast, interlimb parameters (such as step length, double support time), adapted slowly and showed marked after effects (Reisman et al., 2005). These adaptations have been observed to be reduced or slowed in several populations, such as children (Musselman et al., 2011; Vasudevan et al., 2011), elderly (Bruijn et al., 2012c) and patients with severe cerebellar ataxia (Morton and Bastian, 2006), hemispherectomy (Choi et al., 2009), traumatic brain injury (Vasudevan et al., 2014), primary focal dystonia (Hoffland et al., 2014) and Parkinson's disease (Nanhoe-Mahabier et al., 2013; Roemmich et al., 2014).

Here, we explored whether such impairments in locomotor adaptation could be related to a reduced ability to perceive differences in belt-speed ('somatosensory deficits'). Impaired perception could lead to a poorer detection of errors that normally drive adaptation (Bastian, 2011). This is specifically of interest in relation to cerebellar patients. Severely ataxic patients with diffuse cerebellar damage have been observed to demonstrate deficits in locomotor adaptation (Morton and Bastian, 2006) and also in active proprioception (Bhanpuri et al., 2013; Boisgontier and Swinnen, 2014). In an accompanying article (Hoogkamer et al., chapter 4) we evaluated split-belt adaptation in mildly ataxic patients with focal lesions in the cerebellum. Here we address how split-belt adaptation parameters relate to

potential perceptual deficits in these patients. To evaluate how well participants are able to perceive differences between belt speeds we used a perception threshold paradigm that was recently introduced by Lauzière et al. (2014b). In their study the speed of one of the two belts was gradually increased and participants had to indicate when they perceived belt speeds to be different (their perception threshold of locomotor asymmetry). In the group of elderly that was tested, this perception threshold was at a belt speed ratio of 0.88 (belt speed symmetry value of 0.064). During these trials, belt speed ratio was significantly correlated to the asymmetry in stance times (Lauzière et al., 2014b). However, it remains an open question whether one's somatosensory perceptual ability could also explain one's behavior during a split-belt adaptation paradigm, where belt speeds are changed instantaneously, rather than gradually. Indeed, perceptual deficits can be expected to influence several gait parameters where fast adjustments occur, not only stance time but also in limb excursion or swing speed, since these are feedback controlled as well (Hoogkamer et al., 2014a).

During normal (straight ahead) walking humans walk with both symmetric stance times and symmetric limb excursions. During split-belt walking this gait pattern needs to be adjusted to asymmetric belt speeds instantaneously and at least one of those parameters needs to be changed since stance time and limb excursion are inversely coupled through speed. Asymmetries in both limb excursion and stance time have repeatedly been observed (Dietz et al., 1994; Morton and Bastian, 2006; Reisman et al., 2005; Zijlstra and Dietz, 1995). Therefore, it can be expected that a complete symmetry in either stance time or limb excursion will not be reached. Here, we test two opposing hypotheses (Fig. 5.1). The first hypothesis proposes that participants aim to minimize limping during split-belt walking; hence they try to walk with symmetric durations of gait phases (stance symmetry hypothesis). According to this hypothesis, participants who are better able to perceive differences between belt speeds will walk with more symmetry in stance time than participants who are less able to perceive belt speeds differences. Because stance time is inversely coupled to limb excursion during treadmill walking, the participants with best speed-difference perception will walk with the most asymmetric limb excursions. Alternatively, the second hypothesis states that left-right differences in limb excursion are minimized (excursion symmetry hypothesis). This would be in line with the concept that the swing phase is initiated when the hip passes through a particular threshold angle and therefore participants try to reach that angle on both sides of the body. In this case, the participants who are better able to perceive differences between belt speeds will walk with more symmetry in limb excursion than participants who are less able to perceive belt speeds differences. And, simultaneously, the participants with best speed-difference perception will

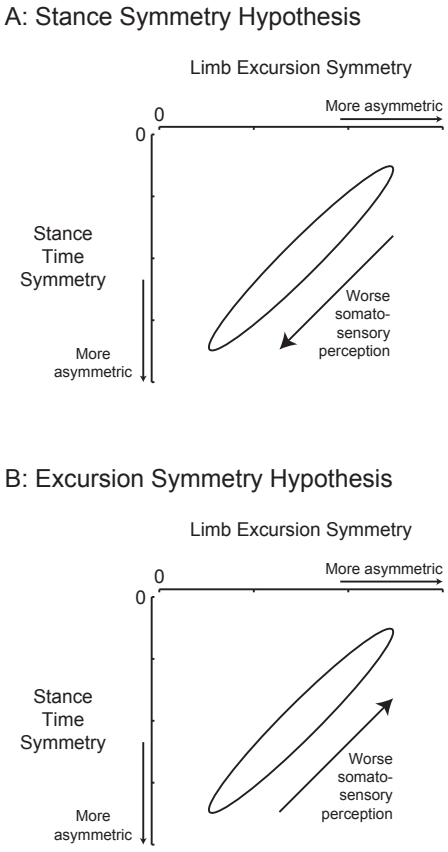


Figure 5.1: ***Two opposing hypotheses for the role of perception of speed differences in early split-belt adaptation. A)*** *According to the stance symmetry hypothesis, participants who are better able to perceive differences between belt speeds will walk with more symmetry in stance time and more asymmetry in limb excursion than participants who are less able to perceive belt speeds differences. The panel shows the expected inversed coupling between Limb Excursion Symmetry and Stance Time Symmetry. Data points in right top corner (small asymmetry in stance times, large asymmetry in limb excursions) would be for participants who are well able to perceive differences between belt speeds; data points in left bottom corner (large asymmetry in stance times, small asymmetry in limb excursions) would be for participants who are less able to perceive differences between belt speeds.* ***B)*** *According to the Limb Excursion Symmetry hypothesis, participants who are better able to perceive differences between belt speeds will walk with more symmetry in limb excursion and less symmetry in stance time than participants who are less able to perceive belt speeds differences.*

Table 5.1: ***Patients were mildly ataxic and in a stable condition (> 5 years post-op)***

#	Age (years)	Time Post-op (years)	Sex	Diagnosis	Interposed Nuclei Lesioned	Adjuvant Therapies Radiation	Adjuvant Therapies Chemo	Lesion Volume (cm^3)	ICARS Total /100	ICARS P&G /34	ICARS Kin Fun /52
1	28.8	13.9	f	Lhermitte Duclos Disease				58.0	3	1	1
2	20.2	8.7	f	Pilocytic Astrocytoma				8.2	3	0	0
3	19.6	11.8	m	Pilocytic Astrocytoma	Left			1.7	6	3	2
4	18.1	6.5	f	Pilocytic Astrocytoma				4.5	1	0	1
5	22.3	17.7	m	Medulloblastoma	Both	Y		22.0	13	6	2
6	18.5	10.0	f	Medulloblastoma	Left	Y	Y	22.6	2	1	1
7	18.6	13.7	m	Medulloblastoma	Right	Y	Y	6.3	19	5	10
8	18.4	15.5	f	Medulloblastoma	Both	Y	Y	5.4	5	1	3

f = female, m = male; Y = yes; P&G = Posture & Gait sub score; Kin Fin = Kinetic Functions sub score.

walk with the most asymmetric stance times. The concept that the swing phase is initiated when the hip passes through a particular threshold angle comes from animal studies (for review of the evidence: Duysens et al., 2000). Observations of prolonged stance phases and gait cycles due to raising the anterior or posterior part of a dog walking on a treadmill (Shik and Orlovsky, 1965) prompted the idea that passing a threshold hip angle could be providing the signal to initiate the swing phase.

5.2 Materials and Methods

5.2.1 Participants

For this study we performed two experiments in two separate sessions. First, fifteen patients with stable focal lesions after cerebellar tumor resection and thirteen healthy participants performed a classic split-belt paradigm. Then, a subset of the participants performed a second experiment: eight patients (age: 20.6 ± 3.6 years ($mean \pm SD$); 5 females, 3 males; Table 5.1) and nine healthy participants (age: 25.9 ± 5.2 years; 6 females, 3 males). The second experiment assessed the perception threshold at which participants could perceive a difference between belt speeds. Patients were in a stable condition (> 5 years post-op; range $6.5 - 17.7$ yrs; Table 5.1) and were able to walk independently (for lesion and therapy details, see accompanying article Hoogkamer et al., chapter 4). We rated severity of ataxia using the International Cooperative Ataxia Rating Scale (ICARS; Table 5.1) (Trouillas et al., 1997). All participants gave written informed consent. The experiments were conducted in accordance with the Declaration of Helsinki and were approved by the local ethics committee.

5.2.2 Split-belt adaptation paradigm

Methods and results for the first experiment are presented in an accompanying article (Hoogkamer et al., chapter 4). In short, the split-belt paradigm started with 3 minutes walking with both belts at 1.0 m/s, followed by ten minutes of walking with one belt at 1.0 m/s and the other belt at 0.5 m/s (SPLIT), ending with five minutes with both belts at 1.0 m/s (Bruijn et al., 2012c). During SPLIT patients walked with the most affected side on the fast belt and healthy controls walked with their non-dominant leg on the fast belt (Morton and Bastian, 2006). Between conditions the treadmill was shortly stopped. Throughout all conditions, kinematics were sampled at 100 samples/s (Vicon Nexus, Oxford Metrics, Oxford, UK). In addition, three-dimensional ground reaction forces and torques were sampled at 1,000 samples/s (instrumented dual-belt treadmill, custom built by Forcelink, Culemborg, The Netherlands).

During all conditions we use 'fast' to refer to gait parameters that were calculated for the leg that was on the fast belt during the SPLIT condition. First, we determined the instants of heel strike and toe-off based on the center of pressure trajectory (Roerdink et al., 2008). Limb excursion was calculated as the distance travelled by the ankle marker in the anterior-posterior direction from heel contact to toe-off of one limb, hence this parameter is overlapping with previously used parameters such as 'stride length' (e.g. Reisman et al., 2005) and 'support length' (e.g. Zijlstra and Dietz, 1995). Reasons for using the new term have been explained previously (Hoogkamer et al., 2014b). Then we calculated symmetry values using (Choi et al., 2009):

$$\text{Limb Excursion Symmetry} = \frac{limb\ excursion_{fast} - limb\ excursion_{slow}}{limb\ excursion_{fast} + limb\ excursion_{slow}}$$

Using a similar equation, we calculated Stance Time Symmetry, using the relative stance time of each leg. In other words, the proportion of stance in the gait cycle was compared for both sides. Early SPLIT values were calculated as the mean value over the first five strides of the SPLIT condition.

5.2.3 Perception threshold paradigm

The second experiment was based on a recently published protocol to assess the perception threshold of locomotor symmetry (Lauzière et al., 2014b). The second session was separated from the first session by at least three weeks, to minimize any transfer from adaptational effects to the second experiment. Participants were fitted with reflective markers on the lateral malleoli and data recording procedures

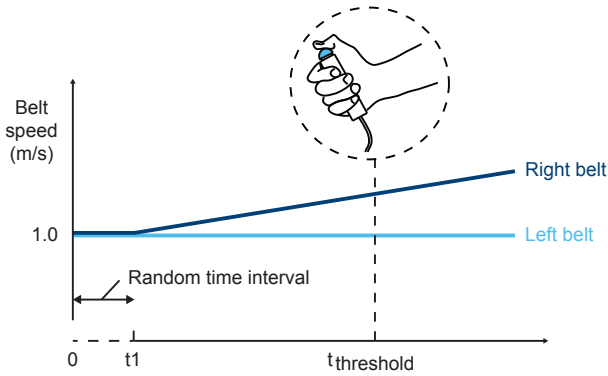


Figure 5.2: ***Perception threshold paradigm.*** *The two belts start at the same speed, and then one belt's speed increases. Participants indicate when they perceive the belt speeds to be different, by pressing a handheld button.*

were similar to those of the first session. During the four experimental trials, the belts started at an equal speed of 1.0 m/s and after a random time interval one of the belt speeds was increased with 0.00278 m/s (0.01 km/h) each second.

Participants had to indicate when they perceived the belt speeds to be different, by pressing a handheld button (Fig. 5.2). Then, they had to indicate verbally which of the belts ran faster. If participants did not perceive any difference the trial was ended after one of the belts had increased speed for two minutes (and ran at 1.33 m/s). To minimize visual and auditory input regarding the speed difference between belts, the participants had to wear industrial ear protection and adapted safety glasses which blocked vision in the lower visual field. In case a participant came close to walking with both feet on the same belt, an experimenter indicated with a pointing gesture that the participant needed to return to the medio-lateral center of the treadmill. In two experimental trials the left belt accelerated, in two other trials the right belt accelerated. In addition we added a sham trial, in which both belts ran at an equal speed of 1.0 m/s for the complete trial duration of 2 minutes. Participants were unaware of the total number of trials they had to perform (i.e. 5), but were given feedback on which of the belts ran faster (if any), after each trial. In between trials, participants walked for two minutes with belts at an equal speed of 1.0 m/s, to wash out any adaptation effects resulting from the (increasing) differences in belt speeds during the experimental trials.

The perception threshold was defined as the symmetry value of the belt speeds at the time that the participant perceived belt speeds to be different (button response),

similar to the definition of the symmetry values of the gait parameters (see above):

$$\text{Perception Threshold} = \frac{Belt\ speed_{fast} - Belt\ speed_{slow}}{Belt\ speed_{fast} + Belt\ speed_{slow}}$$

Belt speeds at the time of the response were assessed by taking the average anterior-posterior velocity of the ankle markers during the preceding stance phase (over the time the heel was on the belt). To minimize effects of learning and differences in initial confidence, only the perception threshold value of the last trial was used.

5.2.4 Statistical analyses

We used Student's t-tests to compare perception threshold values between groups. Linear regression analysis was performed to assess potential correlations between gait parameters during the split-belt paradigm and the perception threshold values from the second experiment. We used a traditional level of significance ($\alpha = 0.05$) for all statistical tests.

5.3 Results

The perception threshold of gait asymmetry appeared on average higher in the patient group than in the control group, but the difference was just below significance level ($6.5 \pm 2.0\%$ vs. $8.8 \pm 3.2\%$; $p = 0.089$). Two cerebellar patients appeared to be less able to detect the speed difference between the belts (outside 95%-confidence interval of the values for the healthy controls; Fig. 5.3 A; P7, P8; Table 5.1). First, we explored whether differences in perception threshold were related to potential asymmetries or other gait parameters during the baseline condition. Perception threshold was not significantly correlated to any of the symmetry values, stride time or stride time variability (all $|r| < 0.40$, $p > 0.10$), but participants with a higher perception threshold did walk with longer relative stance times ($r = 0.54$, $p = 0.026$; Fig. 5.3 B).

Next, we evaluated the relationships between the perception threshold and the Stance Time Symmetry and Limb Excursion Symmetry during Early SPLIT. First, we evaluated the correlation between Limb Excursion Symmetry and Stance Time Symmetry during this episode. The expected coupling between Stance Time Symmetry and Limb Excursion Symmetry was confirmed ($r = 0.82$, $p < 0.001$; Fig. 5.4 A). In addition the correlations between both symmetry values and the perception threshold were significant. For Limb Excursion Symmetry the relation between perception threshold and symmetry was negative ($r = -0.49$,

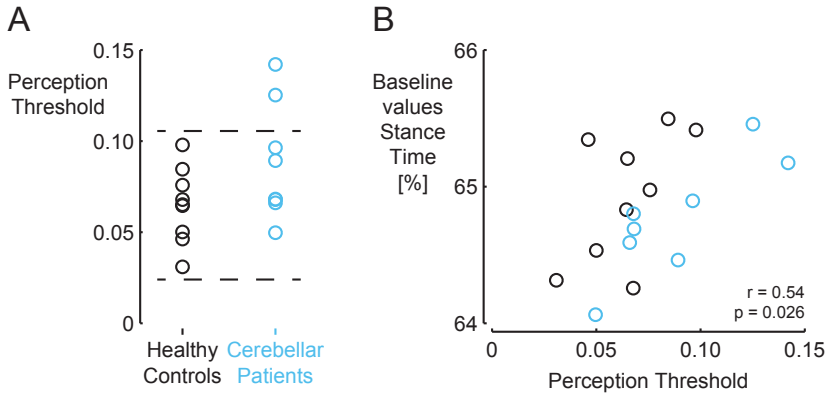


Figure 5.3: ***The perception threshold of gait asymmetry is increased in some patients and correlated to baseline relative stance times.*** ***A)*** *Perception Threshold values for healthy controls (black) and cerebellar patients (blue) are similar, however two cerebellar patients appeared to be less able to detect the speed difference between the belts: values above 95%-confidence interval of the values for the healthy controls (indicated by dashed lines).* ***B)*** *Perception Threshold was correlated to relative stance times during the baseline condition. Values for patients are presented in blue and for healthy controls in black.*

$p = 0.048$; Fig. 5.4 B), indicating that during SPLIT the participants with the highest thresholds initially walked with the smallest asymmetry in limb excursion. Similarly, Stance Time Symmetry was negatively correlated to perception ($r = -0.56$, $p = 0.019$; Fig. 5.4 C), but here the negative correlation indicates more asymmetry for higher perception threshold values. Together these relationships support the stance symmetry hypothesis: participants who are better able to perceive differences between belt speeds will walk with less asymmetry in stance time and more asymmetry in limb excursion than participants who are less able to perceive belt speeds differences.

Furthermore, perception threshold was significantly related to the relative stance time of the slow leg ($r = 0.64$, $p = 0.006$; Fig. 5.5 B), but not to that of the fast leg ($|r| < 0.40$, $p > 0.10$; Fig. 5.5 A). This indicates that the more asymmetric relative stances times in participants with a higher perception threshold were driven by an initial increase in the relative stance time of the slow leg.

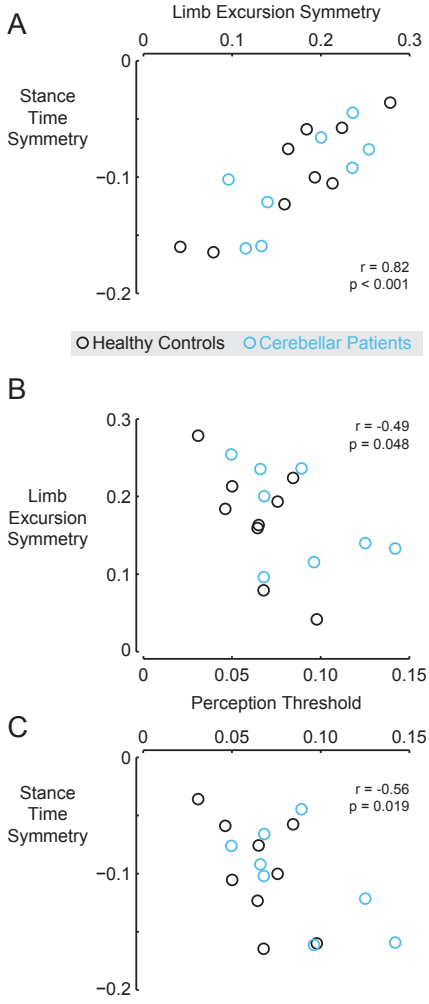


Figure 5.4: ***Observed relationships between speed-difference perception, Stance Time Symmetry and Limb Excursion Symmetry during split-belt walking.*** ***A)*** *Stance Time Symmetry and Limb Excursion Symmetry were significant correlated, as was to be expected based on their mutual coupling to belt speed.* ***B)*** *Limb Excursion Symmetry was negatively correlated to Perception Threshold. Participants better able to perceive belt speed differences (low threshold) walk with less symmetric limb excursions.* ***C)*** *Stance Time Symmetry was negatively correlated to Perception Threshold. Participants better able to perceive belt speed differences (low threshold) walk with more symmetric relative stance times. Values for patients are presented in blue and for healthy controls in black.*

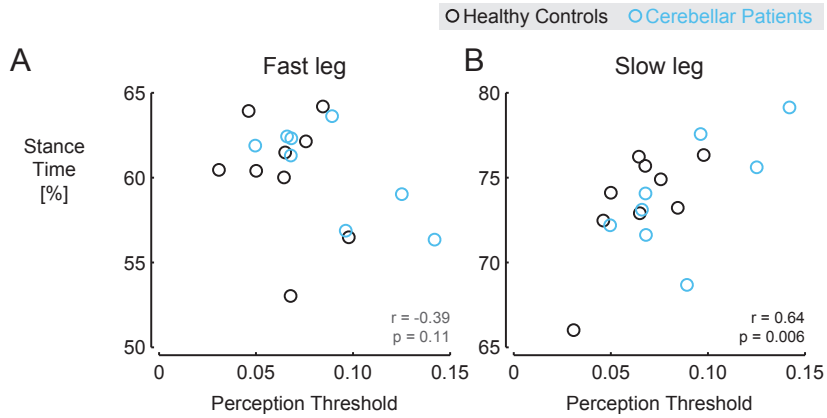


Figure 5.5: ***Correlations between perception threshold values and relative stance times of each leg.*** ***A)*** *Relative stance time of the fast leg was not correlated to Perception Threshold.* ***B)*** *Relative stance time of the slow leg was correlated to Perception Threshold. Values for patients are presented in blue and for healthy controls in black.*

5.4 Discussion

The present study showed that during split-belt walking some participants initially walk with small asymmetry in relative stance times (and simultaneously with large asymmetry in limb excursion), while other participants walk with large asymmetry in relative stance times (and simultaneously with small asymmetry in limb excursion). In addition, it was observed that the participants who initially walked with smaller asymmetry in relative stance times (and simultaneously with larger asymmetry in limb excursion) were better able to perceive differences between belts speeds during the perception threshold paradigm. In particular, it should be noted that perception threshold was significantly correlated to the relative stance time of the slow leg. Participants with worse speed-difference perception initially walked with a disproportionally long stance phase of the slow leg. In contrast, the best participants (in terms of perception) shortened this stance time already adequately during the first five strides of split-belt walking to achieve higher temporal symmetry.

This shortening of the stance phase on the slow side was more prominent than the lengthening of stance on the fast side. This difference between the effects on slow and fast leg can be easily explained by assuming that consciously perceived

differences in speed are more easily translated into shortening of a relatively long stance phase on the slow side than into lengthening a short stance phase on the fast side. This may be related to the size of the time window over which the perception is made. Contact with the belt is a powerful sensory stimulus and it will be present longer when the relative stance duration is longer (such as occurs on the slow side). Hence a shortening of a long stance phase is more easily perceived than a lengthening of a short stance phase. Overall, the present data are in good agreement with Lauzière et al. (2014b), who claimed that Stance Time Symmetry was the main criterion used by their participants to identify the perception threshold. In addition, here the relation with limb excursion is highlighted.

5.4.1 Limb excursion

The present data show that spatial asymmetry (Limb Excursion Symmetry) is relatively more tolerated than temporal asymmetry (relative stance duration) if participants are well able to perceive speed differences. This suggests that during split-belt walking the swing phase is not initiated at a certain hip angle, opposite to expectations based on observations of hip signals being important in the automatic switching of gait phases. In cat studies it was shown that input from hip afferents to the central pattern generators can entrain the locomotor rhythm (Andersson and Grillner, 1983). Such data supported the idea that the swing phase would be initiated when the hip passes through a particular threshold angle (Shik and Orlovsky, 1965). However, experiments on hip joint denervation showed that there is very little effect on gait cycle parameters, thereby supporting the idea that the important hip signal is unlikely to be derived from hip joint afferents (Duysens and Pearson, 1998; Kriellaars et al., 1994). Instead, there is good evidence pointing out that spindle afferent from hip flexors provide the important source of the hip position signal (Hiebert et al., 1996). While these findings were almost all obtained in surgically reduced cat models, it is unknown to what extent this hip signal is important during normal gait. One would expect that hip position is a more tightly controlled variable than the position of other joints. This was investigated in intact cats by measuring these angles under conditions of constrained gait (crouch; Duysens and Pearson, 1998). It was found that cats indeed kept the maximum excursions of hip flexion and extension within stricter limits than the corresponding angles at other joints. However, these data clearly showed that walking is still quite possible even when hip angles deviate strongly from the normal hip threshold angle. This indicates that the hip signal for phase switching can be easily overridden. This may apply to the present work as well. Indeed the data do not support the hypothesis that during split-belt walking the swing phase is initiated when the hip

passes through a particular threshold angle and that therefore participants try to reach that angle on both sides of the body.

All of this suggests that the hip signal for phase switching can be easily overridden. For the cat there is evidence that load receptor input both from extensor muscles and from cutaneous receptors in the foot, is able to reinforce the ongoing extensor activity in the stance phase and delay the ensuing swing phase (Duysens and Pearson, 1998; for review see Duysens et al., 2000). This load feedback may be more important than hip signals for split-belt walking. In this respect it is worth noting that load feedback has been invoked in some of the earliest work in this field to explain the muscle activation patterns in human split-belt walking (Dietz et al., 1994). Later it was also shown that sensory after-effects of split-belt walking are load dependent (Jensen et al., 1998). One can expect that a sudden speed difference between the two sides introduces an imbalance in limb loading. This was indeed observed in kinetic studies. For example, propulsive ground reaction force impulses have been observed to become asymmetric instantaneously during split-belt walking (Ogawa et al., 2012; Roemmich et al., 2014).

In general, changes in stance time and limb excursion symmetry during walking are subject to several additional biomechanical constraints. Retaining a walking gait pattern with double stance phases and without flight phases, limits the asymmetry in stance time. Furthermore, the limb excursion of the slow limb (relative to the stance time of the fast leg) is limited by the maximum swing speed of the slow leg (Bruijn et al., 2012c).

5.4.2 Why do adaptations during split-belt walking take place?

The present data support the idea that at least some of the early adaptation can be driven by perceptual processes. This does not exclude that other factors may be important as well. For example, it has been argued that energy consumption is an important element as well. The question of energy efficiency was already explored by Finley et al. (2013). They observed that improvements in step length symmetry went hand in hand with reductions in metabolic power. In addition, the improvements in step length predicted the size of the reduction in metabolic power, thereby indicating that increasing economy could be a key element driving locomotor adaptation. This process is not mutually exclusive with the presently proposed mechanism.

5.4.3 Role of the cerebellum

These results were obtained on the grouped data of patients and controls since we observed that differences in Stance Time Symmetry and Limb Excursion Symmetry during Early SPLIT were not significantly different between patients with cerebellar lesions and healthy controls (as described in the accompanying article; Hoogkamer et al., chapter 4). Nevertheless, the present data are also informative concerning the expected role of the cerebellum in early adaptation. Notably, the two patients who were less able to detect the speed difference between the belts (Fig. 5.3 A; P7, P8) also had the highest kinetic function ICARS sub-scores (Table 5.1). A role in the (predictive) integration of feedback inputs could explain the increased perception threshold we observed in these patients. This is supported by observations of impaired active proprioception in severely ataxic cerebellar patients (Maschke et al., 2003; Bhanpuri et al., 2013).

In contrast to the studies mentioned above (Maschke et al., 2003; Bhanpuri et al., 2013), we included patients with focal cerebellar lesions who were only mildly ataxic. In general, more localized lesions will result in less prominent deficits. It can be expected that more, or stronger correlations could have been observed when patients were included with more severe ataxic symptoms. For future studies it would be interesting to evaluate how the perception threshold relates to changes in the gait pattern during, and after split-belt walking in patients with more severely affected ataxia (Morton and Bastian, 2006) or stroke (Reisman et al., 2013). Specifically, in the latter patient group it would be of interest to evaluate whether the perception threshold paradigm (Lauzière et al., 2014b) could be used as an assessment tool within split-belt gait retraining programs (Reisman et al., 2013; Hoogkamer et al., 2014a).

To summarize, the present data indicates that participants choose rhythm comfort above spatial symmetry. Presumably, rhythm can be regulated more simply by the central pattern generators for gait when relative stance durations are more equal on both sides. More generally, the tendency of participants to try to obtain symmetry after walking for some time in split-belt conditions has been noted by others as well (Reisman et al., 2005) but not in relation to data on speed-difference perceiving ability. Our data on relative stance phase supports the hypothesis that humans aim to minimize (temporal) limping by using their ability to perceive differences in belt speeds during split-belt walking. The cerebellum may be important in this respect since the deficit in sensory perception of speed differences was typically seen in the most affected cerebellar patients. The present data also indicate that spatial symmetry, in terms of limb excursion, is less tightly regulated with respect

to the sensory perception of speed differences.

5.5 Acknowledgements

We thank Katrien Bastenie and Iris Degrande for assistance during the data collections. This work was supported by Research Foundation-Flanders (FWO grants G.0756.10 and G.0901.11) and The Netherlands Organisation for Scientific Research (NWO grant #451-12-041). JD received a Visiting Professor Grant (CNPq 400819/2013-9). ZP was funded by the European Commission through MOVE-AGE, an Erasmus Mundus Joint Doctorate programme (2011-0015).

General discussion

In this thesis, we focused on the role of the cerebellum in the neural control of gait corrections. Studies in which this role of the cerebellum is explored in patients with well-described lesions are very rare, hence the need for the present work. In the first part, 'Gait stability, cutaneous reflex modulation and the cerebellum' (chapters 1 to 3), we addressed dynamic gait stability and cutaneous reflex modulation. In particular this thesis provides the first systematic study to investigate the role of the cerebellum in the control of reflexes during human gait. In the second part, 'Split-belt adaptation, somatosensory perception and the cerebellum' (chapters 4 and 5), we addressed locomotor adaptation during split-belt walking.

In the first chapter, we performed several experiments in preparation of the main studies on cerebellar patients. We observed that cutaneous reflex modulation patterns can differ substantially between different walking tasks. Specifically, we observed that in backward walking a prominent reflex facilitation occurred in the tibialis anterior muscle of the contralateral leg (with respect to the stimulation), during this leg's stance phase. We expected that these reflexes would be related to the reduced dynamic gait stability of backward walking as compared to forward walking, but reflex amplitudes were not correlated to the overall dynamic gait stability during the backward walking trial. Alternatively, the reflex activity could be related to the phase-dependent function of the tibialis anterior muscle during backward walking. This could not be evaluated in our study due to lack of kinematic data. However, data from another study in our lab (Jansen et al., 2012) indicated that the tibialis anterior muscle slows down the center of mass, which would be

a safe strategy when the sural nerve of the opposite leg is stimulated during its swing phase. Furthermore, the observation that the reflex activity during backward walking was mostly independent of the level of background activity suggested once more that the phase-dependent modulation of cutaneous reflexes has an important supraspinal component.

In the second chapter, we addressed the potential role of the cerebellum within this supraspinal control of cutaneous reflexes. We observed that patients with focal lesions in the cerebellum walked with an overall higher muscle activity in the gastrocnemius medialis and biceps femoris muscles as compared to healthy controls. The increased gastrocnemius medialis muscle activity was accompanied by an overall increased tibialis anterior - gastrocnemius medialis co-activation. Biceps femoris muscle activity was specifically higher during the single stance period. Such antagonist co-activation will increase leg stiffness, which can be a compensation strategy when postural threat is elevated. It is often observed in walking elderly (Franz and Kram, 2013; Hortobágyi et al., 2009; Peterson and Martin, 2010). In ataxia patients with diffuse cerebellar damage, muscle co-activation has been related to the number of self-reported falls per year (Mari et al., 2014). Increased co-activation could theoretically lead to increased reflex activity, due to increased excitability of the motoneuron pool. However, we observed that the phase-dependent reflex modulation patterns of mildly ataxic patients with focal cerebellar lesions were similar to those of healthy controls. This suggested that the cerebellum is not primarily involved in the supraspinal control of cutaneous reflexes. Alternatively, reflexes to self-induced stimuli were not significantly reduced in the patient group, while they were more reduced in the control group. Although the group $\times$ condition interaction was not significant and therefore the interpretation is not straightforward, a reduced inhibition of self-induced reflexes in the patient group would be in line with the known cerebellar role in predicting the sensory consequences of action (for review: Bastian, 2011).

Observations in the third chapter are in line with the hypothesis, forwarded in the previous chapter, that the observed increased muscle activity and co-activation in the cerebellar patients is related to instability of gait. Gait stability was lower in the patient group (λ_S was higher), while the patients were only mildly ataxic (ICARS scores were low and most spatio-temporal gait parameters were similar between groups). Furthermore, λ_S correlated to vermal lesion size ($r = 0.64$; $p = 0.033$), indicating that the patients with the largest lesions in the vermis were the ones with the lowest gait stability. In addition, here it was of interest that the margin of stability was similar between groups, while gait stability was lower in the patient group when evaluated based on λ_S. Since the margin of stability depends on the base of support and since the patients walked with wider steps, it

is likely that the patients used step widening as a strategy to ensure a sufficient margin of stability. This is in line with our observations on backward walking from the first study: healthy controls widened their steps and increased their margin of stability while walking backward. In situations or populations where balance is challenged, step widening can be a safe strategy (Gates et al., 2013; Hak et al., 2013a). However, to what extent this results in under- or overcompensation at the level of the margin of stability is not simple to assess, since a 'sufficient' margin cannot be determined without real gait perturbations. For some populations a small margin can be sufficient, while others might need a larger margin to remain stable following a perturbation. This is in conflict with the notion that a larger margin implies more stability.

In the second part of this thesis, we focused on different aspects of split-belt walking. In recent years there has been evidence that an intact cerebellum is needed to adjust gait to split-belt walking in humans. However, these studies were performed on severely affected patients and they provided limited information on the localization of this function within the cerebellum. First, in chapter 4, we evaluated split-belt adaptation in healthy controls and patients with relatively mild focal cerebellar lesions. We observed that during the split-belt adaptation experiment, patients and healthy controls globally displayed similar changes in gait parameters. However, a group difference was observed in the after-effect of the Stance Time Symmetry: during the early phase of the POST condition, the relative stance times were more asymmetric for the patient group than for the control group. This asymmetry was mainly related to a lower relative stance time of the slow leg in the patient group compared to the control group. Furthermore, during this episode, healthy controls walked with shorter stride times than the patients.

The observation that changes in symmetry of inter-limb parameters, such as step length and relative double stance time, were similar between groups, appears to be in conflict with the study by Morton and Bastian (2006). In the latter study, patients with diffuse cerebellar damage showed deficits in the adaptation of inter-limb parameters, while changes in intra-limb parameters were not affected. The authors considered the fast changes in intra-limb parameters to be reactive, feedback driven and they considered the slow adaptation in inter-limb parameters to be predictive, feedforward driven. Where the former do not require practice and do not produce after-effects (i.e. are not stored by the nervous system), the latter do (Morton and Bastian, 2006). The observation that adaptation of feedforward driven parameters was impaired in patients with diffuse cerebellar damage is in line the known cerebellar role in the predictive control of movement, which we also discussed in chapter 2.

However, two important elements in relation to the study by Morton and Bastian (2006) are often overlooked: only severely ataxic patients (ICARS $>$ 30) were included in their main experiment and they observed that adaptation impairments were specifically related to severity of posture and gait ataxia. The patients with focal cerebellar lesions in our study were only mildly ataxic and as such our findings of unimpaired adaptation of inter-limb parameters in these patients are actually in line with the study by Morton and Bastian (2006), since they also failed to see major effects when ataxia was mild. The relationship between adaptation deficits and ataxia of posture and gait is of particular interest since it suggests that the adaptation deficits could very well be an indirect side-effect of other deficits. In general, adaptation deficits could be related to several different factors. First of all, impaired perception could lead to poorer detection of errors that normally drive adaptation (Bastian, 2011). Second, the adaptation deficit could come from a fundamental deficit in storing new movement patterns. Third, observed deficits in adaption could also be indirect side-effects of other fundamental deficits. The last could specifically be true for split-belt adaptation, where the penalty for not adapting is relatively low. Walking with unadapted inter-limb gait parameters is feasible and penalizes walking economy and stability only to a small extent (Bruijn et al., 2012a; Finley et al., 2013). Along this line, one could argue that for patients with severe balance deficits changing inter-limb coordination has simply no priority as treadmill walking itself is already a very challenging task for them. Not changing intra-limb coordination during split-belt walking would have a larger penalty, since it is physically impossible to walk with both symmetric limb excursions and symmetric relative stances time while belts have different speeds. Taken together these considerations suggest that, next to the cerebellar role in predictive control of movement, other mechanisms could underlie the observed impaired split-belt adaptation in severely ataxic cerebellar patients.

In the final chapter, we tested two opposing hypotheses about the control of split-belt walking. The first hypothesis proposed that participants aim to minimize limping during split-belt walking; hence they try to walk with symmetric durations of gait phases (stance symmetry hypothesis). Alternatively, the second hypothesis stated that left-right differences in limb excursion are minimized (excursion symmetry hypothesis). In line with the first hypothesis, we observed that participants who are better able to perceive differences between belt speeds initially walked with more symmetry in stance time during split-belt walking than participants who are less able to perceive belt speeds differences. Because of the inverse coupling between stance time and limb excursion during treadmill walking, the participants with best speed-difference perception also walked with the most asymmetric limb excursions. In contrast, participants with worse speed-difference perception initially walked

with a disproportionally long stance phase of the leg on the slow belt. The best participants (in terms of perception) could shorten this stance time adequately to achieve higher temporal symmetry. On the slow side the relative stance duration is longer and the sensory input related to contact with the belt could be perceived longer.

The data showed that spatial asymmetry (limb excursion) is relatively more tolerated than temporal asymmetry in participants who are well able to perceive speed differences. However, limb excursion is dominated by hip excursion and from animal studies it is known that hip signals are important in the automatic switching of gait phases and that input from hip afferents to the central pattern generators can entrain the locomotor rhythm (Andersson and Grillner, 1983; Shik and Orlovsky, 1965). Still, some work on constrained crouched gait in cats showed that walking is still quite possible even when hip angles deviate strongly from the normal hip threshold angle (Duysens and Pearson, 1998). Both these observations and our observations from split-belt walking indicate that the hip signal for phase switching can be easily overridden. Here, load receptor input both from extensor muscles and from cutaneous receptors in the foot can be expected to play an important role given its ability to reinforce the ongoing extensor activity in the stance phase and delay the ensuing swing phase in cats (Duysens and Pearson, 1980; for review: Duysens et al., 2000). Similarly, in human split-belt walking, information about loading of the legs has been suggested to affect inter-limb control, based on observations in both the earliest (Dietz et al., 1994; Zijlstra and Dietz, 1995) and more recent studies in this field (Ogawa et al., 2012; Roemmich et al., 2014).

Within the (predictive) integration of these feedback inputs a role for the cerebellum can be expected. In our study with patients with focal cerebellar lesions who were only mildly ataxic, two cerebellar patients appeared to be less able to detect the speed difference between the belts. These two patients were also amongst the most affected ones in our sample. This is in line with earlier observations of severely ataxic patients with diffuse cerebellar damage showing proprioceptive deficits during active movements but not during passive movements (Maschke et al., 2003; Bhanpuri et al., 2013). These deficits in active proprioception are likely related to the cerebellar role in predictive control (Bhanpuri et al., 2013; Boisgontier and Swinnen, 2014) and based on these studies it can be expected that the perception threshold of gait asymmetry would be increased in more severely affected cerebellar patients.

Functional localization

Localization of different gait related functions within the cerebellum could only be approximated in this research, due to several limitations (see below). So far, gait stability had been inferred from measures as step width and stride-to-stride variations (Ilg et al., 2008, 2013). Step width and medio-lateral sway have been observed to be related to fastigial nuclei and inferior posterior vermal damage (Ilg et al., 2008). Step length variability has been observed to be related to lesions in the dorsal dentate nuclei, extending into the interposed nuclei, and to lesions in vermal lobules VIIIa, VIIIb and IX and paravermal lobules V, VI and IX (Ilg et al., 2013). Here, we evaluated dynamic gait stability using the maximum Lyaponov exponent, a valid measure to quantify the ability to recover from small perturbations (Bruijn et al., 2013). We observed that dynamic gait stability was correlated to vermal lesion size, confirming the crucial role for vermal regions in gait stability.

For split-belt walking, we observed that patients who showed the largest after-effects in Stance Time Symmetry were more likely to have lesions in vermal lobules VI and Crus II. Although, these regions are generally not expected to be important in motor control (Stoodley and Schmahmann, 2010), several observations of involvement of these regions in motor tasks have been reported. Vermal lobule Crus II has been related to visuomotor adaptation using cerebellar lesion analysis (Donchin et al., 2012) and to motor learning of eye-hand coordination using functional MRI (Miall and Jenkinson, 2005). Vermal lobule VI has been related to bimanual coordination using functional MRI (Beets et al., 2014; Debaere et al., 2004) and both these vermal lobules have also been observed to be enlarged in well-trained basketball players (Park et al., 2009).

Methodological considerations

Several limitations of the studies within the doctoral project should be addressed. First of all, the different experiments were performed with different subsamples of the same sample of 18 patients with focal cerebellar lesions (Table 2 in General Introduction). As such, all our findings are based on only a relatively small number of cerebellar patients, creating a sampling bias. We aimed to include more patients, but not many patients met our inclusion criteria (age $>$ 18 yrs, focal lesion after tumor resection, $>$ 2 yrs post-op, able to walk independently). For this reason we decided to include patients with accompanying hydrocephalus or additional

radiation and chemotherapy, even though these are sub-optimal conditions for cerebellar lesion symptom mapping (Timmann et al., 2009).

In general, we observed few and rather small behavioral deficits in our patient group. This was related to our inclusion criteria. For lesion symptom mapping, minor, isolated, gait deficits are favorable to reduce the confounding side-effects of more severe gait deficits. However, sometimes to our surprise, patients with substantial cerebellar lesions showed only minor, if any, deficits in the gait characteristics that we measured. This indicates that either the lesioned regions are normally not involved in the control of the characteristics or these regions are normally involved and the central nervous system has learned to compensate their function. Specifically in our patient group such compensation is a likely confounder. Pilocytic astrocytomas often progress rather slowly, which allows for early compensation (Timmann et al., 2009). Furthermore, lesions were most often established at young age when the maturing central nervous system has substantial neural plasticity (Gramsbergen, 2007; Konczak and Timmann, 2007).

Alternatively, one could argue that some of the experiments might have been underpowered to be able to address the small sized deficits that were expected in the patient group. This is specifically of interest for the attenuation of reflexes to self-induced stimuli described in chapter 2. There, no significant group $\times$ condition interaction could be observed, while significant condition effects were observed for more phases of the gait cycle in the control group than in the patient group. However, in the same experiment we did observe significant group differences in muscle activity during the unstimulated steps (for the gastrocnemius medialis and biceps femoris muscles). Therefore, we argue, not sample size, but rather the heterogeneity of the patient group might have reduced the statistical power of the different experiments. Focal lesions with a heterogeneous distribution within the cerebellum are theoretically ideal for lesion symptom mapping. However, potentially confounding factors as additional radiation or chemotherapy and neural plasticity (mentioned above), together with increased intra-individual behavioral differences in the healthy population, reduce the power of such lesion symptom mapping. Specifically, inter-individual variation in cutaneous reflexes is often observed to be large, even among healthy control participants (chapter 1). These high inter-individual differences in reflexes among the healthy controls make appropriate lesion symptom mapping analysis impossible (chapter 2).

Finally, voxel-based lesion symptom mapping has several methodological limitations. Specifically, for sample sizes smaller than $n = 20$, patients need to be classified in subgroups with unaffected and affected behavior. Ideally, behavioral outcomes of these subgroups are clearly separated, with the affected patients' behavior

substantially different from the behavior of the healthy controls and the unaffected patients. However, classification of subgroups is much less straightforward for smaller sample sizes and for behavioral outcome measures which show substantial inter-individual differences within the healthy control group (Hoogkamer and Meyns, 2014). Another limitation of lesion analysis is that regions that are important for specific functions might not be identified because few if any of the patients have lesions in these regions.

Future research directions

Starting from the insights obtained in this doctoral project, more specific questions can be addressed in future studies. To confirm our observations which strongly suggest that the cerebellum is not primarily involved in phase-dependent modulation of cutaneous reflexes during gait, it would be useful to evaluate more severe ataxic patients, such as patients with degenerative cerebellar diseases. Such research could also give more insights into the role of the cerebellum in suppression of cutaneous reflexes to self-induced stimuli. However, it is important to note that gait pattern and muscle coordination in these patients might be severely affected, thereby introducing a large degree of variability. As such, it might be less feasible to assess the direct effects of the cerebellar damage on reflex modulation, as it might be confounded by other deficits or compensation strategies. Here, it could be useful to include control experiments where healthy controls mimic the deviations in the gait pattern of the cerebellar patients (for instance increased co-activation), to assess how much changes in reflexes are related to changes in the gait pattern or primarily to the cerebellar damage.

From our study on gait stability in cerebellar patients (chapter 3) it became clear that still a lot can be learned about this topic. We showed that in mildly ataxic patients, λ_S is a more sensitive measure of gait deficits than gait variability. However, for several specific features of ataxic gait, such as increased step width and muscle co-activation, it is unclear to what extent they result from deficits or from compensation strategies. Specifically, we argued that the margin of stability and step width should not be used as mutually independent measures to classify gait as stable or instable. From a biomechanical perspective, the margin of stability from the 'extrapolated center of mass' concept (Hof et al., 2005) is a valid parameter to indicate gait instability. However, in the stable range, an increasing number of studies (e.g. Hak et al., 2013b; chapters 1 and 3) showed that a larger margin of stability does not always correlate to more stable gait conditions, experimentally induced or as quantified by more sensitive stability

measures. Those margin of stability results are likely to be confounded by step widening strategies of the participants (see above). To gain more insights in compensation strategies, relations between gait stability, muscle co-activation, step width and the margin of stability should be evaluated in longitudinal studies in patients with acute cerebellar damage, both in the initial recovery phase and over time. This will provide insights into how the central nervous system learns to compensate for specific cerebellar-related gait deficits. In addition, assessing these gait parameters pre and post rehabilitation interventions could give information on which compensation strategies and interventions have the best effects on gait stability and on the reduction of fall prevalence.

The validity of the maximum Lyapunov exponent (λ) for assessing the stability of gait was already discussed in the General Introduction. In this thesis we now can add that λ_S is a sensitive measure of gait deficits in mildly ataxic cerebellar patients. For our stability study (chapter 3) we quantified λ_S for the medio-lateral displacement of pelvis. To prevent any confounding effects of gait velocity, all trials were performed on a treadmill at a standardized walking speed of 1.0 m/s. Furthermore, the treadmill enabled us to analyze 150 consecutive strides. It may be argued however, that treadmill walking remains part of an artificial environment. In this respect it is worth noting that recently it was shown that λ_S, quantified by accelerometry during short bouts of walking in daily-life activities, helped to prospectively identify individuals at risk of falls (van Schooten et al., 2014). This strong finding supports the potential of the Lyaponov exponent. Future work should be aimed at further validating and applying this measure during daily-living activities and in simple clinical settings (without the need for a fully equipped gait laboratory).

Additional insights into compensation strategies and gait stability could come from studies on cerebellar patients where normal gait is perturbed. Gait perturbations using virtual stepping stones (Potocanac et al., 2014) or unexpected loss of ground support during walking (van der Linden et al., 2007) will provide more insights into the cerebellar role in feedforward control of gait.

Since our patient population (focal lesions due to tumor resection) displayed only few and rather small behavioral deficits, for future studies it is important to include either patients with more severe forms of ataxia or a larger group of patients with a comparable ataxia severity. A larger patient sample would allow for more powerful voxel-based lesion symptom mapping analyses, as discussed above. In large samples it is possible to analyze behavioral data continuously, without the need of a cut-off value. For this thesis, availability of patients was limited, but future projects could apply a multi-center approach. Such a multi-center approach would specifically be

useful for a longitudinal study design, such as discussed above. Evaluation of gait characteristics during the initial recovery phase and over time, for all acute lesion patients in multiple centers, will provide valuable insights into how the central nervous system learns to compensate for specific cerebellar-related gait deficits, specifically when these behavioral measures could be combined with MRI analyses such as lesion symptom mapping or network analyses (see below).

Alternatively, or in addition, other groups of patients with more severe forms of ataxia could be studied. For example, this could be achieved by studying patients with focal lesions due to subacute or chronic stroke or by using voxel-based morphometry for patients with degenerative cerebellar damage. Again, it should be noted that observations in such studies could be confounded by other deficits or compensation strategies. Furthermore, stroke patients are often of advanced age which could also confound behavioral outcomes in split-belt studies (Bruijn et al., 2012c).

As mentioned above, lesion symptom mapping analyses in the patient group with focal lesions after tumor resection are likely to be confounded by compensation and reorganization in the brain. Here, more complex brain imaging analysis techniques could provide valuable insights. For instance, graph-theoretical network analysis of neural networks (Bullmore and Sporns, 2009) could be used to determine how cerebellar pathology alters the functional and structural network connections of the cerebellum with the rest of the brain, and whether these connectivity changes are associated with locomotor deficits. This method combines functional (resting state) and diffusion MRI data. It can be expected that networks involving the supplementary motor area, the secondary somatosensory area and parietal-(pre)motor regions will show an increased functional and structural connectivity in the cerebellar patient population. Functional connectivity is quantified by network topology properties such as the level of network connectivity, clustering behavior and network cohesion, length of communication pathways, connection strength between brain regions, and hub formation. Structural connectivity can be assessed by applying white-matter tractography between motor regions using Diffusion Tensor Imaging (DTI) data.

Finally, it would be interesting to evaluate how the perception threshold relates to changes in the gait pattern during and after split-belt walking in patients with more severely affected ataxia (Morton and Bastian, 2006) or stroke (Reisman et al., 2013). Although we do not fully support all conclusions in the introducing paper by Lauzière et al. (2014b), we think their paradigm will help to further understand the control of split-belt walking as we argue in this thesis (Hoogkamer et al., 2014a; see Appendix B of this thesis). With their work on the perception threshold of

locomotor symmetry, Lauzière et al. (2014b) introduced a very promising new paradigm. This paradigm will not only provide insights in the prevalence of functional asymmetries in specific patient populations such as individuals with hemiparesis (Brière et al., 2010; Patterson et al., 2010), it also adds an interesting new perspective in understanding how split-belt adaptation is driven. The paradigm has potential to help in understanding why specific populations adapt slower to split-belt walking (e.g., the elderly; see Bruijn et al., 2012c) or even in assessing which patients are most likely to benefit from split-belt gait retraining (Reisman et al., 2013). Insights into the factors which do (or do not) drive locomotor adaptation are crucial for optimization of gait rehabilitation strategies. The new 'perception threshold of locomotor symmetry' paradigm provides such insights. To build on these insights it is important to consider all potential factors related to afferent input from the limbs.

Conclusions

This doctoral thesis addressed the role of the cerebellum in dynamic gait stability, in cutaneous reflex modulation during gait and in locomotor adaptation, all important features of neural control of gait in non-steady state conditions. Our data suggests that the cerebellum is not primarily involved in cutaneous reflex modulation but that it could act in attenuation of self-induced reflex responses. Furthermore, the cerebellum is important in the control in dynamic gait stability. Finally, we observed that during split-belt walking, mildly ataxic patients and healthy controls globally displayed similar changes in gait parameters. However, the after-effect of asymmetric relative stance times was most pronounced in the patient group. In addition, we observed that participants who were less able to perceive differences between belt speeds, initially walked with more asymmetric stance times during split-belt walking. Together the findings in this thesis illustrate the major benefit that can be obtained by integrating multiple disciplines (biomechanics, neurophysiology, neuroimaging) in acquiring new knowledge on the function of the cerebellum in human gait.

A

Terminology and definitions in split-belt walking

Published in Gait & Posture as:
Hoogkamer W, Bruijn SM, Duysens J. Stride length asymmetry in split-belt locomotion. *Gait Posture* 39: 652-654, 2014.

Abstract

The number of studies utilizing a split-belt treadmill is rapidly increasing in recent years. This has led to some confusion regarding the definitions of reported gait parameters. The purpose of this paper is to clearly present the definitions of the gait parameters that are commonly used in split-belt treadmill studies. We argue that the modified version of stride length for split-belt gait, which is different from the standard definition of stride length and actually is a measure of limb excursion, should be referred to as 'limb excursion' in future studies. Furthermore, the symmetry of stride length and stride time is specifically addressed.

Highlights

- We explain the calculation of spatial gait parameters for split-belt gait
- The definition of stride length in split-belt gait is problematic
- We argue that this measure should be referred to as 'limb excursion'
- Using standard definitions, stride time can never be asymmetric when not turning
- Similarly, stride length can never be asymmetric when not turning

Keywords

Gait; limb excursion; split-belt walking; symmetry; treadmill walking

A.1 Introduction

Split-belt treadmills are used to study locomotor adaptation. After pioneering work in the 80 and 90ties, focusing on whether walking with two legs at different speeds is possible, this work has seen a huge boost recently (Fig. A.1). A large part of this boost has come from the group of Amy Bastian, who explored locomotor adaptation, and its practical implications, in rehabilitation (Reisman et al., 2005; Torres-Oviedo et al., 2011). In recent years ever more labs are using a split-belt to study gait adaptability. However, as the number of studies increases, definitions used for different gait parameters have become less clear in a substantial number of cases.

From our experience in discussing split-belt research with clinicians, biomechanists and students, we see that concepts are often unclear, as a result of poor understanding of commonly used gait parameters, and the impossibility of applying these parameters to split-belt gait. Specifically, the definition of stride length in split-belt gait and its (a)symmetry is counterintuitive.

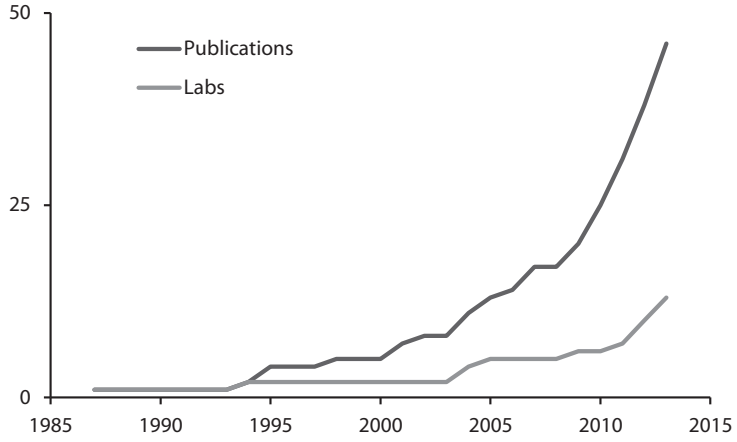


Figure A.1: ***Overview of the total number of split-belt publications and authoring labs.*** *Only studies on split-belt gait in humans where belts run on different velocities were included.*

A.2 Paradoxical asymmetries

In split-belt studies, but also in studies on pathological gait, left-right asymmetry of gait parameters is often reported. It should be noted, however, that if we assume straight-ahead gait, some gait parameters can exhibit asymmetric behavior, while other variables, according their common definitions cannot, irrespective of whether participants are healthy or not. This may only change when these parameters are defined differently. Specifically, as 'stride' commonly refers to a complete gait cycle, both stride time and stride length should be symmetric as they are summations of right and left step times and lengths, respectively. However, introduction of several alternative definitions of stride length and of asymmetry has resulted in mentioning asymmetric stride times and lengths (Bayat et al., 2005; Nanhoe-Mahabier et al., 2013; Okada et al., 2011; Reisman et al., 2005). Such alternative definitions are not necessarily problematic, but when these definitions are not clearly addressed, the use of alternative definitions will result in confusion. For example, if we consider a stride length asymmetry of 1.4 and a stride length of 0.8 m (both in the range of earlier reported values for split-belt gait: Bruijn et al., 2012c; Nanhoe-Mahabier et al., 2013; Reisman et al., 2005) and would interpret stride strictly as a complete gait cycle, we would erroneously think the participant is turning (Fig. A.2). This example already illustrates that it is good practice to not only report (a)symmetry values, but also values of the actual gait parameters, so that readers can judge the basis of the (a)symmetry.

The purpose of this paper is to clearly present the definitions of split-belt gait parameters and discuss the implications thereof. Furthermore, we advocate the use of 'limb excursion' as an alternative for 'stride length' in split-belt research.

A.3 Spatial parameters in treadmill gait

One commonly applied method to calculate spatial gait parameters during treadmill gait is based on the timing of gait events combined with belt speed. Stride length is equal to stride time multiplied by belt speed; step length is equal to step time multiplied by belt speed. Note that both length measures should be corrected for displacements in the global frame of reference, as these calculations are only precise when subjects do not drift on the treadmill and when foot placement relative to the center of mass is constant. The latter will not be the case in asymmetric gait with different swing speeds between limbs (see the work of Roerdink et al. (2007, 2012)).

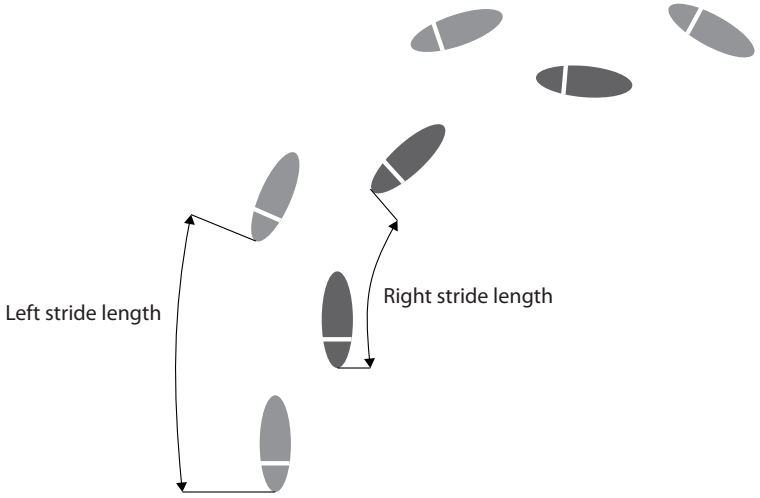


Figure A.2: ***Top view of foot placement in case of asymmetric stride lengths, which is only possible when turning.***

To avoid problems with displacements on the belt, spatial parameters can be calculated based on the positions of the feet (Reisman et al., 2005). In this case step length is best determined based on toe positions just before lift-off, because in healthy gait at this gait event the toes of both feet are on the floor. More often step length is estimated based on the inter ankle distance at initial contact (Reisman et al., 2005), which slightly deviates from the overground definition of step length, as the heel of the trailing limb is already raised at this time. Stride length is then calculated as the summation of left and right step length.

Unlike normal treadmill gait, in split-belt gait, temporal parameters and belt speeds cannot be used easily to calculate spatial parameters, as both belts run at different speeds. Thus, spatial parameters need to be calculated from feet positions directly. For step length, this is done by taking the anterior-posterior distance between the ankle marker of each leg at initial contact of the leading leg. Fast step length refers to the step length measured at initial contact of the fast leg (leg which makes contact with the fast moving belt); slow step length refers to step length measured at slow leg initial contact (Reisman et al., 2005) (Fig. A.3). It is important to calculate these distances at initial contact, as the feet will move closer together (or apart) during double support, since the belts move at different speeds (Fig. A.3).

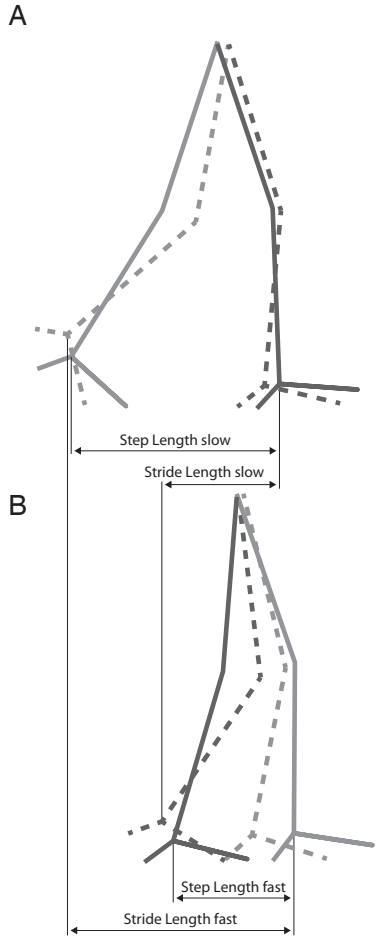


Figure A.3: ***Definitions of spatial gait parameters for split-belt gait. A)*** *Side view during initial contact of the slow leg (full lines) and during lift-off of the fast leg (dashed lines).* ***B)*** *Side view during initial contact of the fast leg (full lines) and during lift-off of the slow leg (dashed lines). A 'fast step' occurs when moving from the dashed lines in panel A to the full lines in panel B, while a 'slow step' occurs when moving from the dashed lines in panel B to the full lines in panel A. The interval between initial contact of the leading leg (full lines) and the lift-off of the trailing leg (dashed lines) consists of the double support phase. Step length is calculated by taking the anterior- posterior distance between the ankle marker of each leg at initial contact of the leading leg; fast step length refers to the step length measured at fast leg initial contact and slow step length refers to step length measured at slow leg initial contact. Stride length is calculated as the distance traveled by the ankle marker in the anterior-posterior direction from initial contact to lift-off of one limb.*

A.4 Stride length or limb excursion

For stride length, the situation is more complex, since stride length includes both left and right steps. Logically, one would define stride length as the sum of left and right step lengths, similar to normal gait. However, in their influential paper on split-belt adaptation Reisman et al. (2005) introduced a modified version of stride length, which is calculated as the distance travelled by the ankle marker in the anterior-posterior direction from initial contact to lift-off of one limb (Fig. A.3). The advantage of this method is that it allows assessing asymmetry in 'stride lengths' between fast and slow legs. However, this type of stride length is not comparable to 'normal' stride length, which can never be asymmetrical on a treadmill (c.f. Fig. A.2).

Instead, this definition of 'stride length' is closer to a measure of limb excursion, or distance travelled during stance. While the group who introduced this measure (Reisman et al., 2005) moved away from the use of stride length and focused on other spatial parameters based on limb excursion (Malone et al., 2012), we, and other groups, initially adopted their definition of stride length to be consistent (Bruijn et al., 2012c; Nanhoe-Mahabier et al., 2013). However, we have to acknowledge that stride length is not the most appropriate name for this gait parameter, since in any other condition 'stride' refers to a complete gait cycle. Alternatively, we argue that in future research this measure is best referred to as 'limb excursion'; this to avoid any confusion with 'true' stride length.

Furthermore, limb excursion can easily be related to other excursion measures that previously have been shown to be important in the control of gait, such as limb angles at initial contact and lift-off (Malone et al., 2012) and hip excursion (Duysens et al., 2000). For instance, from work in cats it is well-known that the hip extension at lift-off is a crucial factor in the automatic switching from stance to swing (Duysens et al., 2000). Hence it is conceivable that subjects automatically try to maintain these hip excursions at a relatively constant level in order to profit from this automatic switching. Thus, the use of limb excursion measures, including limb angles at initial contact and lift-off may lead to novel insights in the control of gait.

A.5 Conclusions

We strongly advice to report gait parameters for both sides and not just symmetry values. Additionally, when using ambiguous gait parameters, we encourage

addressing their definitions and calculation procedures clearly to prevent wrong interpretations. Lastly, the use of 'limb excursion' measures for split-belt gait should be preferred over stride lengths, since this nomenclature is more correct, and such measures may lead to novel insights.

A.6 Acknowledgements

We thank Dr. M. Roerdink for helpful suggestions and stimulating discussions. This work was supported by Research Foundation-Flanders (FWO; grant G.0.756.10). SMB was supported by a grant from the Netherlands Organisation for Scientific Research (NWO #451-12-041) and an FWO-grant (G.0901.11) to JD.

Gait parameters affecting the perception threshold of locomotor symmetry

Published in Perceptual & Motor Skills as:
Hoogkamer W, Bruijn SM, Duysens. Gait parameter affecting the perception threshold of locomotor symmetry: comment on Lauzière, et al. (2014). *Percept Mot Skills* 119: 474-477, 2014.

Abstract

In a recent work on locomotor symmetry while walking on a split-belt treadmill, Lauzière and co-workers determined the perception threshold of gait symmetry in a sample of healthy elderly. In addition, they aimed to determine which particular gait parameters affect the symmetry of the perception threshold. Although only temporal and kinetic gait parameters were measured (and no kinematics), it was suggested that stance time symmetry is an important criterion that participants use to identify the threshold. Here it is argued that several other gait parameters could qualify equally well as main criteria used to identify the threshold and that these parameters should be taken into account in future studies.

B.1 Introduction

With their work on the perception threshold of locomotor symmetry, Lauzière et al. (2014b) introduced a very promising new paradigm. This paradigm will not only provide insights in the prevalence of functional asymmetries in specific patient populations such as individuals with hemiparesis (Brière et al., 2010; Patterson et al., 2010), it also adds an interesting new perspective in understanding how split-belt adaptation is driven. The paradigm has potential to help in understanding why specific populations adapt slower to split-belt walking (e.g., the elderly; see Bruijn et al., 2012c) or even in assessing which patients are most likely to benefit from split-belt gait retraining (Reisman et al., 2013).

Insights into the factors which do (or do not) drive locomotor adaptation are crucial for optimization of gait rehabilitation strategies. The new perception threshold of locomotor symmetry paradigm provides such insights. To build on these insights it is important to consider all potential factors related to afferent input from the limbs. Here it is argued that alternatives exist for stance time symmetry, which was suggested by Lauzière et al. (2014b) to be the main criterion used by the participants to identify the threshold. Specifically, since limb angle and hip angle at heel-strike and toe-off have been shown to be important in the control of gait in both humans (Malone et al., 2012) and cats (Duysens et al., 2000), limb excursion symmetry and swing speed symmetry are important candidates which should be considered in future studies aimed at identifying gait parameters important in optimizing gait rehabilitation strategies.

Lauzière et al. (2014b) appropriately analyzed the gait parameters measured in their study. A significant correlation was observed between stance time ratio (slow belt/fast belt) and perception threshold (expressed in belt speed ratio) both at the time of perception of (a)symmetry. Furthermore, it was stated that most participants (75%) reported to have used 'temporal information' to judge their locomotor symmetry. Based on these observations it was postulated that stance time symmetry is the criterion that participants use to identify the threshold.

B.2 Temporal and spatial components

As indicated in the study, the correlation between stance time ratio and the belt speed ratio at perception threshold is a direct result of the close link between stance time ratio and belt speed ratio, in general. It has been observed repeatedly that stance time ratio changes simultaneously with belt speed ratio (Reisman et al.,

2005, 2007, 2013). However, it should be noted that other gait parameters change similarly in response to changes in belt speed ratio (Reisman et al., 2005, 2007). All parameters that change instantaneously and are proportional with changes in belt speed ratio will display a high correlation with the belt speed ratio at perception threshold. This is specifically true for limb excursion (or 'stride length'; Hoogkamer et al., 2014b), a spatial gait parameter closely related to stance time. In fact, in our own laboratory, a regression analysis on belt speed ratio and limb excursion ratio data from similar experiments confirmed the strong relationship between belt speed ratio and limb excursion ratio (unpublished observations).

With regard to the question concerning what kind of information the participants used to judge their locomotor symmetry, Lauzière et al. (2014b) grouped the subjective answers into three main categories. Here it should be noted that answers classified as 'temporal information' also included answers related to the speed of the leg. Indeed, speed of the leg has a temporal component, but simultaneously a spatial component as well.

B.3 Afferent feedback

In a broader perspective, the observations reported by Lauzière et al. (2014b) suggest a role for temporal information in perception of locomotor asymmetry, but these observations do not rule out that other information might be (more) important. Although temporal cueing has often been observed to have a beneficial effect on gait characteristics (e.g. Roerdink et al., 2007; Zanotto et al., 2013), from a neuromechanical perspective an important role for position and stretch information can be expected. In cats, stretch of hip flexors has been observed to be an important trigger for the onset of flexion at the end of the stance phases (for review: Duysens et al., 2000). In humans, hamstring activity at the end of the swing phases appears to be controlled by the stretch of these muscles during this phase; as such this is a self-regulating system since a high swing speed requires more braking activity in the hamstrings (for review: Duysens et al., 1998). Similarly, several other possibilities have been suggested for afferent feedback in split-belt adaptation. These alternatives range from load sensors (Jensen et al., 1998) to symmetry of joint moments (Roemmich et al., 2012), joint work (Roemmich et al., 2014) or total external mechanical work (Thajchayapong et al., 2014). In principle, these elements could also affect the perception of locomotor symmetry or asymmetry (note that the threshold evaluated during the ascending series is related to perception of asymmetry, while the threshold evaluated during the descending series is related to perception of symmetry). With respect to the suggested importance of load

receptors it is worthwhile noting that it has been shown that the positive work at the ankle joint of the fast leg has been observed to be related to changes in belt speeds between legs (Roemmich et al., 2014).

B.4 Conclusions

Taken together, Lauzière et al. (2014b) have introduced an interesting paradigm, with potential to help in understanding how split-belt adaptation is driven and how gait rehabilitation strategies can be optimized. In addition to stance time symmetry, which is related to belt speed ratio at perception threshold due to its coupling to belt speed ratio, other gait parameters should not be neglected when attempting to identify gait parameters important in locomotor adaptation. From a neuromechanical perspective, spatial (limb excursion symmetry), hybrid (swing speed symmetry) or work related (positive ankle joint work) gait parameters are suitable candidates and valuable insights can be gained when these are considered in combination with the perception threshold paradigm.

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Appositions

- The metabolic cost of uphill running is not simply equal to the sum of the cost of level running and the cost of performing work to lift the body mass against gravity.
- Backward walking is less automated than forward walking and requires special control circuits.
- In relation to running injuries, changing your foot strike pattern from rear foot to forefoot striking (or vice versa) is probably more hazardous than the biomechanical disadvantages of persisting with your habitual foot striking pattern.

Curriculum vitae

Wouter Hoogkamer was born February 24^{th} 1982 in Lisse, the Netherlands. After finishing his secondary education at Fioretti College in Lisse in 2000, he started his studies in Civil Engineering at TU Delft. For his Master's thesis he studied vibrations in deep-water risers under supervision of Prof. Dr. Andrei Metrikine. After earning his Master's degree he worked some years in industry as an engineer. Due to the lack of scientific challenges in his work, Wouter made an important switch in his career and enrolled in the pre-master program at the Faculty of Human Movement Sciences at the VU University of Amsterdam. During his studies he worked as a teaching assistant for Dr. Knoek van Soest and Dr. Mirjam Pijnappels. For his results in the pre-master program Wouter was awarded the G.J. van Ingen-Schenau Promising Young Scientist Award, which allowed him to do his Master's thesis in the locomotion lab of Prof. Dr. Rodger Kram at the University of Colorado, in 2009. Wouter graduated cum laude in 2010 and then moved to Belgium to start his doctoral training at the KU Leuven. Under supervision of Prof. Dr. Jacques Duysens he studied the neural control and biomechanics of human walking. His PhD-project on the role of the cerebellum in reactions to gait perturbations was co-supervised by Prof. Dr. Stephan Swinnen, Prof. Dr. Frank van Calenbergh, Prof. Dr. Stefan Sunaert and Dr. Sjoerd Bruijn. This thesis is the result of the work performed within this project. Next to his academic activities, Wouter is a former 400m/800m runner and current middle distance running coach at Daring Club Leuven Atletiek.

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Hoogkamer W, Bruijn SM, Duysens J. Relations between split-belt adaptation, after effects and perception of gait asymmetry. 7^{th} World Congress of Biomechanics, July 2014, Boston, MA, USA.

Hoogkamer W, Bruijn SM, Swinnen SP, Van Calenbergh F, Duysens J. Split-belt adaptation in cerebellar patients with focal lesions. ISPGR 2014 Congress, June 2014, Vancouver, BC, Canada.

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Hoogkamer W, Bruijn SM, Van Calenbergh F, Duysens J. Dynamic gait stability in cerebellar patients. XXIV Congress of the International Society of Biomechanics, August 2013, Natal, Brazil.

Hoogkamer W, Potocanac Z, Uniszkiewicz D, Dierckxsens S, Swinnen SP, Duysens J. Early rate of split-belt adaptation is correlated to somatosensory perception. 2^{nd} Joint World Congress of ISPGR and Gait & Mental Function, June 2013, Akita, Japan.

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Hoogkamer W, Van Calenbergh F, Swinnen SP, Duysens J. The role of the cerebellum in cutaneous reflex modulation and self-induced reflex attenuation. 10^{th} Belgian Society for Neuroscience meeting, May 2013, Brussels, Belgium.

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Dankwoord

Ten eerste wil ik alle mensen bedanken die zo vriendelijk zijn geweest om als proefpersoon deel te nemen aan mijn studies: vooral degenen met cerebellaire laesies, maar natuurlijk ook alle controle proefpersonen. Voor sommigen was het een behoorlijke afstand om naar Leuven te komen, desondanks hebben velen van jullie deelgenomen aan meerdere meetsessies. Hartelijk dank om zo veel moeite te doen ten bate van de wetenschap in het algemeen en dit project in het bijzonder.

Verder moet ik natuurlijk niet overdrijven, uiteindelijk is dit boekje gewoon het resultaat van mijn functie als wetenschappelijk onderzoeker. En terwijl men bij iedere baan baat heeft bij hulp van collega's en steun van vrienden en familie, is dit een van de weinige functies waarbij je kans hebt een uitgebreid dankwoord te schrijven. Ik zal het daarom kort houden.

Eerlijk gezegd was er eigenlijk maar één echte reden waarom ik naar Leuven verhuisde. Het project werd begeleid door Jacques Duysens. Ik volgde slechts het advies van een van mijn docenten in Amsterdam, maar het bleek een gouden tip. Jaak, het was me een groot genoegen om met u te mogen werken, ik heb enorm veel van u geleerd en ik hoop dat u ondanks uw emeritaat nog lang in de wetenschap actief zal blijven!

Ook mijn co-promotoren hebben dit project zeker naar een hoger niveau gebracht. Ik wil Frank Van Calenbergh bedanken voor zijn inzet bij het recruteren en de klinische analyse van de proefpersonen. Ook de ondersteuning bij de medische beeldvorming en de analyse daarvan van Stefan Sunaert en zijn team waardeer ik

erg. Hoewel zij formeel geen co-promotor waren, hebben ook Stephan Swinnen en Sjoerd Bruijn veel bijgedragen aan dit project (wat duidelijk blijkt uit het feit dat hun namen op ongeveer alle artikelen staan die in dit boekje gebundeld zijn).

Furthermore, I would like to thank all my colleagues from the Movement Control & Neuroplasticity Research Group. Specifically, Pieter and Zrinka, not just for sharing the office with me, but much more for being my soundboard, always ready to let me keep you from your work. Dank ook aan Inge voor de hulp bij de MRI analyses en aan Sonja voor de administratieve ondersteuning bij mijn 'bizarre' dossier. Also, many thanks to the thesis students for their valuable input in the project and for bringing inspiration. Furthermore, I want to thank Rodger, Benedicte and Toon for keeping the sport science fire burning in me.

Dan wil ik mijn atletiekvrienden in Nederland en België noemen en mijn familie, voor hun steun in wat ik doe. Rory, voor altijd in mijn gedachten. Ten slotte, last but not least, Linda, al lange tijd mijn soulmate en sindskort zelfs mijn vrouw: voor je onvoorwaardelijke steun, je jou zijn en natuurlijk je uitmuntende editting capaciteiten! Wij horen bij elkaar.

Dit project is financieel ondersteund door Fonds Wetenschappelijk Onderzoek - Vlaanderen (FWO: G.0756.10). Het drukken van dit boekwerk is financieel ondersteund door:

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